JPRS 82224

12 November 1982

East Europe Report

SCIENTIFIC AFFAIRS

No. 759

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CONTENTS

TEXTBOOK COVERS CHEMICAL, BIOCHEMICAL METHODS OF ANALYZING TOXIC AGENTS

West Berlin LEHRBUCH DER MILITAERCHEMIE in German 1977 (signed to press 15 Feb 76) Vol 2 pp 299-400; 403-480

[Chapters 28 and 29 from "Textbook of Military Chemistry. Vol 2: Sabotage and Plant Poisons, Detoxification and Detoxification Agents, Analysis of Chemical Warfare Agents and Poisons," by Maj Siegfried Franke, chemical engineer (chapters 14-19, new version of chapters 20-23); Peter Franz, chemical engineer (chapters 24-33); Prof Dr Gerhard Gruemmer (collaboration on chapters 17-19); and Lt Col Werner Warnke, chemical engineer (chapters 20-23), VEB Military Publishing House of the German Democratic Republic. A translation of the tables of contents and chapters 14, 15, and 16 from this textbook is published under the heading, "Textbook Covers Toxic Agents, Decontamination," in JPRS 81435, 3 Aug 82, No 750 of this series, pp 2-53]

[Text] 28. Chemical Methods for Analysis of Toxic Agents

In this chapter we describe chemical analysis procedures which make it possible qualitatively and quantitatively to determine warfare agents and sabotage toxins in the various sample materials. The methods vary in terms of their capacity (selectivity, sensitivity, accuracy, time requirement, range of application) and so do the requirements which have been established for the analyst's skill level and the laboratory's equipment.

The analysis and determination methods are essentially described in the sequence customary in the practical implementation of warfare agent analysis in field laboratories. This arrangement of the analytical part, which deviates from the arrangement of Volume I, is practical also for other reasons. If we had followed the pattern used in Volume I, the necessarily separate treatment of the analysis system involved in skin-damaging and throat-irritating arsenic warfare agents would have restricted the clarity of the presentation.

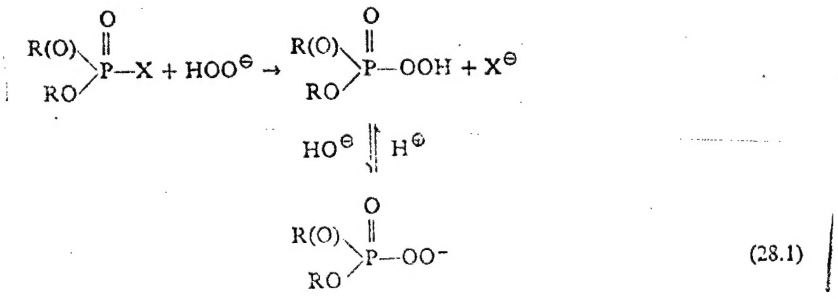
28.1. Organic Phosphorus Compounds

The fact that toxic warfare agents and the most important insecticides are to be found among the representatives of this group of compounds led to a constant search for ever more sensitive analysis and determination methods, as we can see from the wealth of literature available on this subject.

28.1.1. Qualitative Analysis Methods

28.1.1.1. Schoenemann Reaction

This reaction of organic phosphorus compounds, which is still used today in many instances, was discovered in Germany by Schoenemann (21) in his work on trilon warfare agents back in 1944. It is based on the idea that, during the reaction of phosphorus organyls with hydrogen peroxide, intermediary peroxyphosphoric acids are formed in the alkaline medium which, in an alkaline solution are in a state of balance with the perphosphonatanion which quickly oxidizes certain aromatic amines into stained products. According to investigations by Larsson (22), this conversion can be explained in analogy to alkaline hydrolysis in terms of a nucleophile attack of the perhydroxide ion upon the P = O bond. The actual formation of a peracid was proven by Gehauf (23) through the oxidation of thiosulfate into sulfate by means of the reaction mixture; in the meantime, Hilgetag has isolated peroxyphosphoric acid esters.



(28.1)

It has been established that the reaction of the phosphororganyl with hydrogen peroxide takes place about 50 times faster than alkaline hydrolysis. This observation can be explained in terms of the intermediary formation of a hydrogen bridge between the perhydroxide ions and the P = O bond as a result of which the dissociation of the P-X bond is made easier. This speed difference can be clearly seen in Table 28.1 given by Marsh and Neale (24).

Table 28.1. Stability of Sarin in Connection with Various pH Values and with the Addition of Hydrogen Peroxide (temperature 25° C)

Buffer	pH Value	Half-life ($t_{1/2}$ in hrs	
		Without H_2O_2	With 0.1% H_2O_2
HCl/KCl in water/acetone (1:)	1.2	1.7	1.7
Aqueous acetate solution	5	6.7	6.3
Aqueous bicarbonate solution	7.4	8.0	1.4
Aqueous phosphate solution	8.4	1.4	0.2
Phosphate in water/acetone (1:1)	8.4	2.5	0.5
Phosphate in water/acetone (1:1)	11.0	6.5	1.8 (seconds)

Composition of phosphate buffer: 0.5 m K_3PO_4 + 0.01 m KH_2PO_4.

This table also shows the ineffectiveness of hydrogen peroxide in an acid environment. Similar to the differences in the hydrolytic stability of the phosphororganyls, there is also a difference in the speed of the conversions of various compounds with the perhydroxide ion. This is to be shown clearly by three second-order speed constants (at 25° C) given by Marsh and Neale (24) for various types of organic phosphorus compounds.

Sarin	$0.94 \cdot 10^5$ 1 · mol^{-1} min^{-1}
Tetraethyl pyrophosphate (TEPP)	$2 \cdot 10^3$ 1· mol^{-1} min^{-1}
Paraoxon	14 1· mol^{-1} min^{-1}

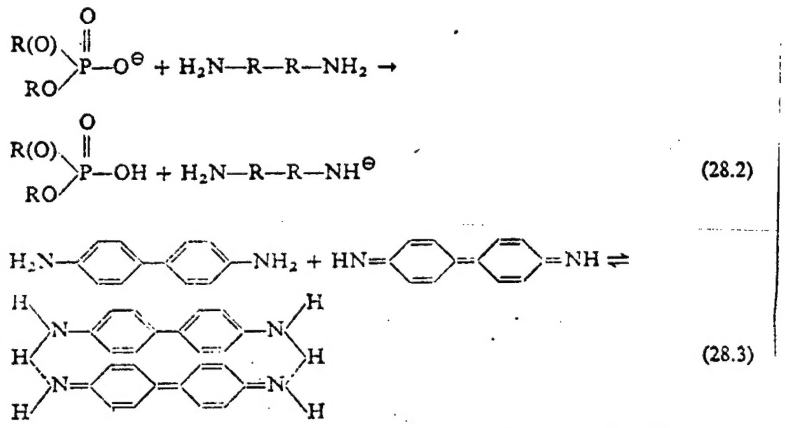
In the light of these values we can estimate that the sensitivity of the reaction in case of application to paraoxon is not great and that indeed is the case. On the other hand, in case of phosphoroganyls with low hydrolytic stability or when we work in the more strongly alkaline range (above pH 12), hydrolysis appears with a disturbing effect as a competitive reaction. It is thus not possible to determine the easily hydrolizable dihalogenides of the phosphoryl compounds or of the fluorphosphonic acid cholinesters with the help of the Schoenemann reaction. In contrast to that, when we use the very slowly hydrolyzing V-substances [mass-destruction warfare agents], measures are necessary to speed up the reaction, for example, an increase in the temperature to a maximum of 40° C. A temperature increase by 10° C increases the speed of the reaction between the phosphororganyl and the peroxide by about factor of 2.5.

In general we can say that, to achieve maximum sensitivity, the conditions during the reaction must be adapted to the phosphororganyl which is to be analyzed. One can keep the pH value low to suppress the hydrolysis but one must then accept an increase in the time until the staining maximum has been reached. This considerable extension in terms of time--which above all takes place in conjunction with slowly reacting compounds, such as DFP, can be made up through an increase in the hydrogen peroxide concentrations but the latter must not be selected so high that there will be a noticeable oxidation of the amine alone due to the peroxide. The optimum conditions are as follows:

pH value	9-9.5
Peroxide concentration	0.01-0.1%
Amin concentration	4 mol/mol phosphororganyl.

We will now turn to the second part of the Schoenemann reaction, that is, the oxidation of the amines. So far, nothing has been published on the kinetics of this reaction; concerning the type of developing reaction products, there are various, hitherto not reliably proven interpretations. Marsh and Neale (24) noted that the absorption spectra of the oxidation products of o-dianisidine in the case of the Schoenemann reaction agree with Sarin and that the oxidation products agree with the alkaline ferric cyanide solution or with potassium permanganate but that on the other hand there are deviations in the properties of the staining products (extractability, change in stain when acid is added). The authors assume that the dye here is a complex made up of quinone diimine and unaltered amines. The origin of quinone diimine can be explained in terms of the prior formation of semiquinone imine when a proton

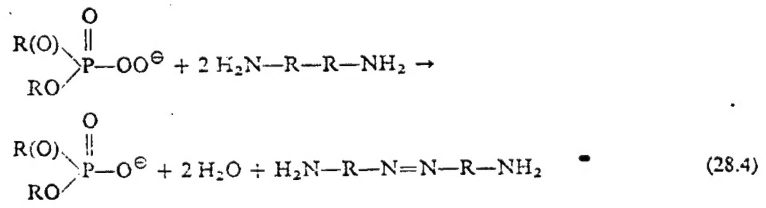
of the amine base is given off to the phosphorus-containing radical. The semiquinone imine radical, which is unstable in alkaline solution, then either forms quinone diimine due to the loss of a second proton to another phosphorus-containing radical or diimine and the original amine will develop due to the disproportioning action of two molecules.



(28.2)

(28.3)

The formation of an azodye stuff is another possible explanation of the staining products.



(28.4)

It has been found (22) that the absorption spectrum of the oxidation product of benzidine is very similar to the spectrum of diaminoazobenzol but was not identical to it. Aksnes and Sandberg (25) oxidized benzidin and 0-dianisidine with peracetic acid and investigated the oxidation products. In the case of benzidine, they found 4-amino-4'-ditrodiphenyl as the only oxidation product and in the case of o-dianisidine, on the other hand, they found three products:

I. 3,3'-dimethoxy-4-amin-4'-nitrodiphenyl (yellow, λ_{max} 370 nm)
II. 3, 3'-dimethoxy-4-amino-4-nitrosodiphenyl (brown, λ_{max} 445 nm)
III. Bis (3,3'-dimethoxy-4-amino)-azodiphenyl (red, λ_{max} 435 nm).

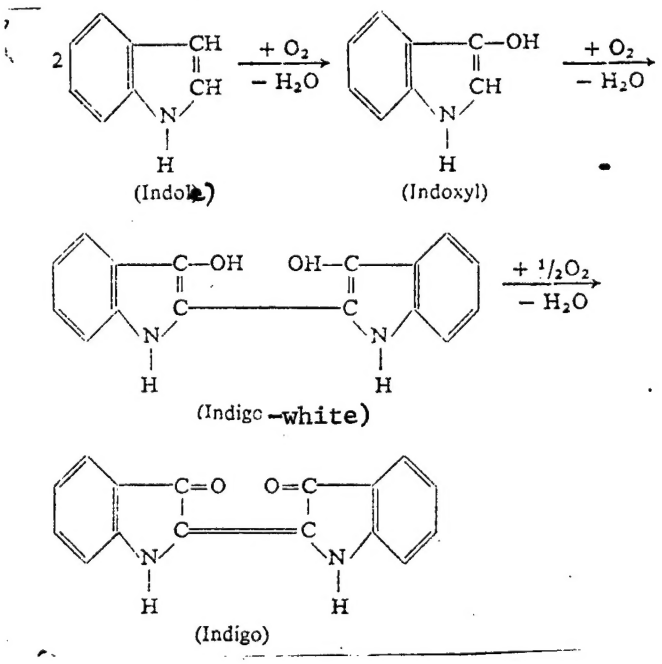
Compound 3 would seem to be the reaction products between the nitroso compound II and o-dianisidine.

In addition to the previously mentioned aromatic diamines called benzidine and o-dianisidine, we can also use o-tolidine for the colorimetric performance of the Schoenemann reaction. As the oxidation agent we use, in the simplest case, an alkaline hydrogen peroxide solution but some peracid compounds, such as socidum proborate, sodium pyrophosphate peroxide, and urea peroxide

are stabler and by virtue of their partly alkaline reaction eliminate the need for the additional use of a buffer.

Acetone is especially suitable as solvent; it reveals a sensitivity-increasing effect which so far has not been clearly explained. The less volatile solvent hexanediol-(1,6), isopropanol, diethylphthalate, and tetraline are better suited for the absorption of the warfare agents in washing bottles but in that case, about 10 percent acetone should be added to the reaction mixture.

Concerning the practical performance of the reaction it is to be noted that, quite naturally, the sequence and the manner in which the reagents are combined will decisively influence the sensitivity. The best results in quantitative determinations are obtained when the sample solution is added drop by drop to the freshly prepared mixture of the reagents. When we add the reagent to the sample solution, we must either add the phosphororganyl sample to a mixture of alkaline peroxide solution with the amine or we must add the alkaline peroxide solution to the mixture of the sample solution with the amine. Under most circumstances may peroxide be added before amine. Perphosphonic acid would react with the peroxide surplus and would here be decomposed along with the development of oxygen. The result would be a considerable sensitivity loss. An increase in sensitivity is achieved if, instead of the previously mentioned amines, we add indole (26) whose oxidation at pH values of around 9 via heavily green-blue fluorescingindoxyl and indigo-white leads to blue indigo.



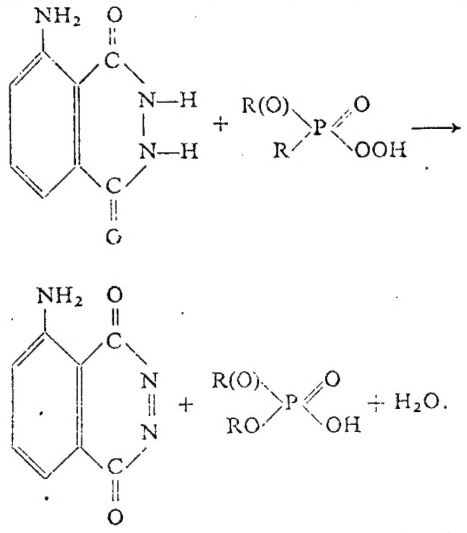
(28.5)

The peroxyphosphoryl compound here causes oxidation all the way to indoxyl; the oxidation continues rather easily due to the oxygen from the residual peroxide. The presence of indoxyl was proven through the addition of isatin to the reaction mixture and the formation of indirubin which was observed in the process; its illustration in this fashion was described in the literature on the subject.

In addition to the measurement of the fluorescence, the blue color of indigo is also analyzed in this modification of the Schoenemann reaction, for example, in the American indicator vials for nerve gases. For a more convenient measurement of the fluorescence, it is possible to stablize the fluorescing products by adding acetone of glycerine.

Another modification of the Schoenemann reaction is the evaluation of the green-blue chemical luminescence which appears upon oxidation of luminol (3-aminophthalic acid hydrazide) (28).

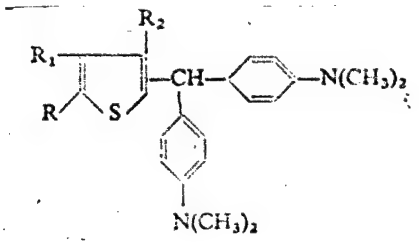
In the investigations conducted by a Yugoslav research group, it was found that chemical luminescence in the case of Sarin reveals two maximums, the first of which briefly takes us back to the above reaction while the second, longer-lasting maximum is caused by the oxidation of luminol through the oxygen developed during the reaction of perphosphonic acid with excess peroxide (16). The addition of alkali halogenides increases the sensitivity, presumable due to the intermediary formation of hypohalogenite (31).



(28.6)

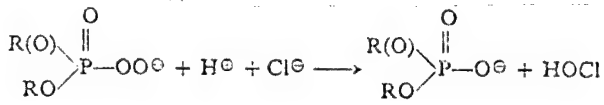
The boundary concentration (about 0.5 µg/ml) is higher than in the case of the fluorescence modification. Tabun can be determined with good sensitivity (0.1 µg/ml) through a similar method, using the chemical luminescence indicator luzigenin (N,N'-Dimethyldiacridiniumdinitrate) (32). The measurement of the weak chemoluminescence light in case of quantitative determinations is possible with secondary electron multipliers.

Here it is interesting to take a look at the work of a Canadian research team whose members, searching for redox indicators suitable for the Schoenemann reaction, encountered some triaryl methane compounds which have a high extinction coefficient and favorable redox potential (-0.7 to 1.0 V) (29).

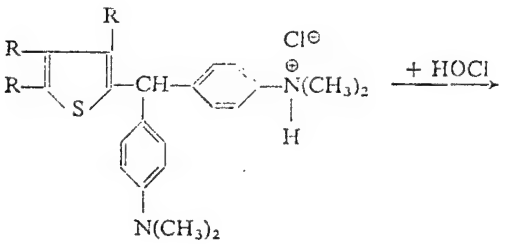


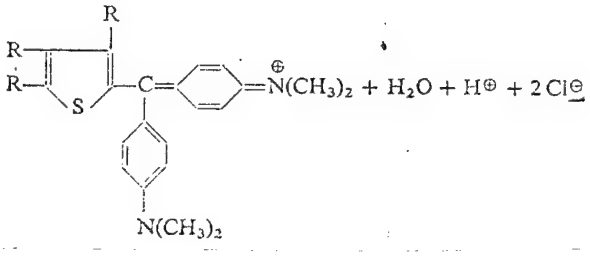
(28.7)

The best suited compound was made with R = R_1 = CH_3 and R_2 = H 4,4'-4,5-dimethyl-2-thenylidene-bis-N,N-dimethylanilin. An interesting aspect in the case of these indicators is the fact that the oxidation, in contrast to the performances of the Schoenemann reaction described so far, must take place in an acid environment and that the presence of chloride ions is therefore necessary. It is therefore assumed that the indicator is oxidized by hypochloric acid which develops during the acidification due to the reaction of the peroxyphosphoryl with the chloride ion.



(28.8)





(28.9)

We therefore work in the following manner: the phosphororganyl is allowed to react for about 30 sec with an alkaline peroxide solution (pH 10.5) and we then add a solution of the leuco compound of the indicator in diluted sulfuric acid containing sodium chloride. With a pH value of 1.6, we then, up to a boundary concentration of 0.1 µg Sarin/ml, get a blue stain which is better suited for visual recognition than the yellow-orange coloration obtained when we use o-Dianisidin. Tabun produces negative results, probably due a reduction to the peroxyphosphororganyl ions due to the cyanide ions in alkaline solution.

The Schoenemann reaction is a group reaction. In addition to the phosphororganyl, all compounds, which give us peroxide compounds with hydrogen peroxide, react here. Thus we see that aldehydes, acid halogenides, acid anhydrides, and

arylsulfonylhalogenides reveal a similar reaction. This can be used for the determination of these substances, for example, the acylcholin compounds or diphosgene and in connection with their employment as model substances. Thus, p-chlorobenzolsulfonylfluoride is used as a slightly toxic standard substance for the plotting of calibration curves and benzolsulfochloride is a good imitation agent for Sarin.

On the other hand however we must watch out for the disturbing influence of these compounds, especially the aldehydes contained in alcohols and in acetone; this disturbing influence must be eliminated prior to the use of these solvents for the analysis and determination of the phosphororganyls by means of special purification methods.

A similarly disturbing effect also springs from some cations which under certain circumstances are present in water samples; these are cations, such as copper, iron, and manganese; they must be masked by adding complex forming agents such as sodium hexametaphosphate. Disturbances due to heavily oxidizing and reducing decontamination agents must be prevented by means of corresponding preliminary treatment of the sample.

The Schoenemann reaction is used in indicator tubules [vials] for Sarin, in the American nerve gas detectors, in warfare indicators, and in various analysis and determination methods in portable and mobile field laboratories. The range of application of the Schoenemann reaction also extends to the analysis and determination of insecticide phosphoric acid esters, among others, Systox, DDVP, Dipterex, Diazinon, Phosphamide, Rogor, and Dimethoate.

We will confine ourselves to the following description of two analysis methods because we can naturally also use for the analysis the methods given for quantitative determination (see Section 28. 1.2.1.).

Staining Reaction (23)

Reagents:

Solution A (2.5-percent solution of benzidine in acetone)
Solution B (0.25-percent solution of sodium perborate in water, solution must be made fresh everyday!)

Operating Procedure:

To a mixture of 0.5 ml of solution A and 2 ml of solution B we add 2 ml of sample solution in i-propanol or water. Organic phosphorus warfare agents become visible by means of a yellow stain that reaches its maximum after about 20 minutes.

Boundary Concentration

The boundary concentration is at 1-2 µg/ml. The sensitivity can be increased if the stain is shaken out with benzene, toluene, or xylene. When we use an alcoholic sample solution, we must, prior to extracting the dye, dilute with a water. It is a good idea simultaneously with the sample to set up a

blank test with the same quantities of reagents and i-propanol or water and to observe the results.

Spot Reaction (332)

Reagents:

Solution A (0.02 g o-dianisidindihydrochloride are dissolved in 10 ml of methanol and 15 ml of water. To this we add five drops of 5 N sulfuric acid and 4 ml of 25-percent hydrogen peroxide solution. The reagent is stable for 12 hours.)

Solution B (potassium-sodium-carbonate, 3-percent in water).

Operating Procedure

One drop of solution A is placed in the depression in a spot [drop plate] and is mixed with one drop of solution B. After adding a drop of the sample solution, a red stain reveals the presence of organic phosphorus warfare agents of the type of Sarin.

The spot [drop] reaction can also be performed on filter paper.

Boundary Concentration:

The recording limit is at 0.1-0.5 µg.

Fluorescence Reaction (26)

Reagents:

Solution A (indole, 1-percent in aceton)
Solution B (sodium perborate, 25-percent in water)
Solution C (glycerin, 1-percent in water).

Operating Procedure:

A mixture of 2 ml of solution A, 1 ml of solution B, 3 ml of solution C, and 3 ml of acetone is added to 1 ml of sample solution and is immediately observed in the light of an UV lamp. A blue-green fluorescence reveals the presence of organic phosphorus warfare agents in the sample solution.

Boundary Concentration

The boundary concentration is at 0.05-0.1 µg/ml. In concentrations of more than 1 µg/ml, the solution is stained blue-green after a short time. Because of its significance in the success of the analysis, we will now describe the special purification of the solvents and amines which are needed for the Schoenemann reaction.

Acetone

Acetone is treated with potassium permanganate for the oxidation of the aldehydes. We add about 4-5 g $KMnO_4$ for 1 lit and allow the mixture to boil for several hours in the reflux. After that we filter and the acetone is distilled, in connection with which we discard the first 5 percent of the distillate down to a residue of 5 percent.

Alcohols

To eliminate the aldehydes, the alcohol is boiled for 1 hour in the reflux after addition of 1 g benzidine or o-dianisidin for 1 lit and it is then distilled. After adding 1 g oxalic acid, we distill once again. All solvents used are in each case tested for their suitability for the Schoenemann reaction by setting up a blank test which must not reveal any coloration.

Amines (33)

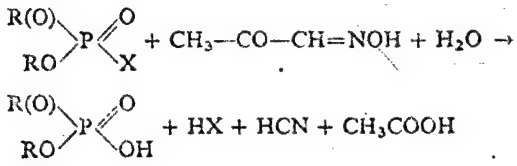
We dissolve 50 g of the raw amine (benzidine, o-tolidine, or o-dianisidin) in a mixture of 35 ml of 85-percent hydrazine hydrate and 500 ml of 95 percent ethanol. Then we add 10 g powdery activated carbon and 6 g sodium bisulfite and we heat and stir for another 2 minutes. The hot solution is filtered and the filtrate is mixed with 1.5 lit of cold, distilled water. The precipitated white amine is filtered off, it is washed with 100 ml of water, and it is dried in the vacuum exsiccator over phosphorus pentoxide. The amine should look white, otherwise the purification process must be repeated.

o-Dianisidindihydrochloride (34)

Industrial o-dianisidinbase is purified according to the above directions and by means of repeated recrystallizing from ethanol, adding activated carbon, until the substance is white. We dissolve it, through cooling in ice, until saturation in acetone and, amid constant cooling, we introduce dry hydrogen chloride gas. The precipitating hydrochloride is filtered off, it is washed with cold acetone and it is then dried. After that it is ground into a powder and once again washed with acetone. The substance must look pure white and must be kept in a bottle made of brown glass or in the dark.

28.1.1.2. Reaction with Oximes

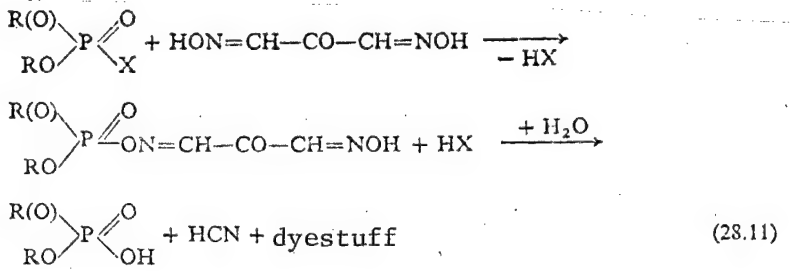
During studies on the reaction between various oximes and organic phosphorus compounds, which were conducted during the search for suitable decontamination agents and antidotes in the United States and Great Britain, several analytically usable conversions [reactions] were also found. Saville (35) found that monoisonitrosoacetone can quickly and completely hydrolyze certain organic phosphorus compounds through nucleophile attack upon the phosphorus atom and is decomposed into hydrocyanic and carboxylic acid.



(28.10)

Other acylizing compounds, such as acid halogenides, also react in an identical manner. By converting the hydrocyanic acid formed with chloramin-T into cyanogen chloride and its staining reaction with pyridin-pyrazolon (36), it is possible to determine phosphororganyls colorimetrically.

Sass, Fischer, and their associates (37, 38) found that, in the reaction of phosphororganyls with diisonitrosoacetone, a red-violet polymethin dyestuff of hitherto unexplained structure is formed.



(28.11)

The optimum pH value for this reaction is 8.3-8.6; in this range, we also reach a maximum in the staining in connection with Sarin within 7 minutes. When we use monobasic salts of diisonitrosoacetone (sodium salts or monoamino-salts of dibutylamine or guanidine) we do not have to add a buffer solution for the adjustment of the desired pH value. The advantage of this reagent resides in the easy handling and the manifold application possibilities. For example, monoamino-salts or multibasic sodium salts can be made into tablets or they can be filled into gelatine capsules to evidence organic phosphorus compounds in water. Using penta-sodium salt, we can make analysis pins for determination in air. The quantitative determination is made by measuring the stain in the colorimeter at 486 mn or 580 mn.

Analysis with Diisonitrosoacetone (37)

Reagents

Diisonitrosoacetone solution, 0.4 percent in water; buffer solution of pH 8.4 (to 500 ml of 0.1-molar boric acid, we add about 17 ml 0.5-molar caustic soda or we use a freshly prepared 2-percent sodium bicarbonate solution).

Operating Procedure

To 1 ml of acqueous or alcoholic sample solution we add 1 ml diisonitrosoacetone solution and 3 ml of buffer solution. Organic phosphorus warfare agents are indicated by a red-orange to red-violet stain.

Boundary Concentration:

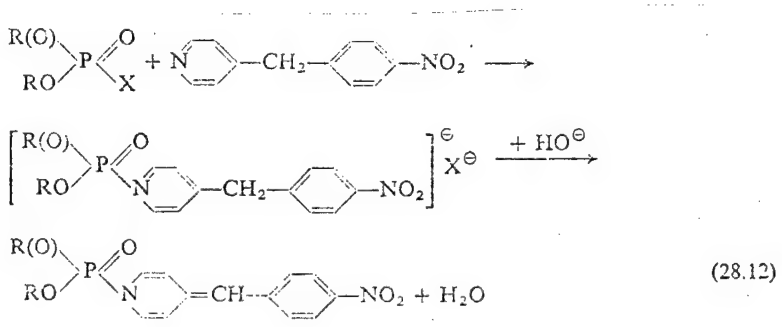
Sarin, Soman, Tabun, and DFP are detected here up to a boundary concentration of 0.25-1 μg/ml.

Irregularities

Methylphosphoryldichloride, phosphoroxychloride, and similar compounds, acylating compounds, such as acetic anhydride, as well as benzene sulfochloride and benzoylchloride under corresponding conditions produce identical stains. These substances will not cause any disturbances in field tests but in handling water samples it is necessary to watch out for the absence of cations (iron (II), copper) and free halogens which if necessary must be masked or eliminated.

28.1.1.3. Reaction with 4-(p-Nitrobenzyl)-pyridin (p-NBP)

This reagent very generally reacts with alkylating compounds, including many warfare agents and with phosphorylating compounds (39). According to a reaction whose course is assumed as follows, p-NBP, with some organic phosphorus warfare agents, such as Sarin, Soman, and Tabun, forms a blue stain whose absorption maximum is at 625 nm (40).

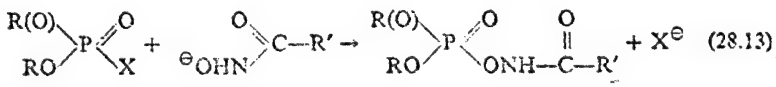


(28.12)

In spite of its disadvantages, which reside in the low specificity and the low sensitivity in the case of phosphorus-containing warfare agents (25-75 μg/ml), we can say that this analysis method in field analysis is important as a preliminary test for the presence of toxic agents. The analysis is performed along the lines of the methods described in connection with the determination of nitrogen yperites (see Section 28.3.2.2.).

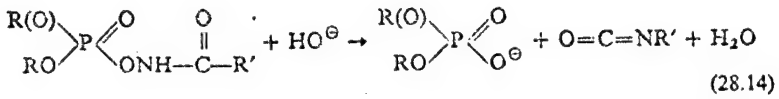
28.1.1.4. Reaction with Hydroxamic Acids

In this reaction, which takes place in a slightly alkaline medium, there is furthermore--after polarization of the P = O bonds--a nucleophile substitution reaction of the hydroxamic acid anion.

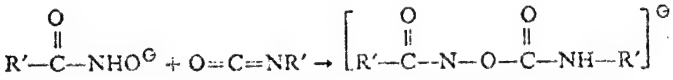


(28.13)

This unstable phosphorylation product is decomposed amid Lossen transposition [rearrangement] (41) into isocyanate which reacts with another hydroxamic acid ion toward the corresponding carbamyl hydroxamate.



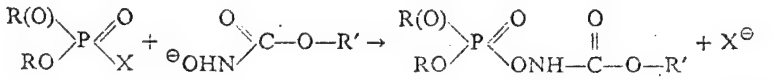
(28.14)



(28.15)

The reaction with benzhydroxamic acid yields phenylcarbamylbenzhydroxamate, which thermally can be decomposed into anilin. In the acid medium, we get an azomethin dye due to reaction with p-dimethylaminobenzaldehyde so that, due to the course of this reaction, for example, Sarin can be detected up to a boundary concentration of 0.4 mg/ml (42).

If the phosphororganyls at pH 8 are transformed with N-Hydroxy-arlycarbamates, then stable phosphorylation products are formed (43) which can be precipitated by means of the acetification of the reaction mixture and which can be used to identify the organic phosphorus compound.



(28.16)

The illustration of these derivatives can be accomplished in an aqueous medium or, in case of slightly hydrolyzing compounds, in pyridine. To a solution of 1 g N-hydroxyphenylcarbamate (0.0065 mol) in 50 ml of water, we add 0.045 mol of the phosphororganyl compounds. The pH value is kept at 8 through the corresponding addition of 0.2 normal caustic soda. If no further acid formation can be observed, we acetify with hydrochloric acid (pH 3) and we filter the white precipitate. After drying and recrystallizing, we determine the melting point. In case of lighter water-soluble phosphorylation products we extract after acetification with chloroform, we dry with magnesium sulfate, and after restriction through the addition of Ligroin (boiling point up to 80° C), we precipitate. The melting points of the phosphorylation products of some organic phosphorus toxins are as follows:

Sarin 137-139" C, recrystallized from methylenechloride/Ligroin
DFP 122-124° C, recrystallized from chloroform/Ligroin
TEPP 65-66° C, recrystallized from chloroform/petroether.

28.1.1.5. Detection of VX

For the special protection of organic phosphorus warfare agents of type V, there are two possibilities in addition to the use of general methods:

(a) Detection of thiol sulfur, mostly after hydrolytic cleavage of the molecule;

(b) Detection of tertiary nitrogen in the S-diisopropylaminoethyl group with Dragendorff's reagent.

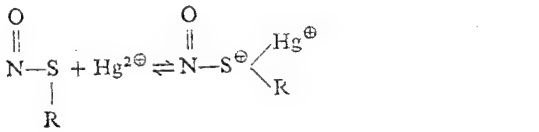
The two detection methods, described below, are particularly useful in the sensitive determination of thiol sulfur. The first detection method is a modification of a colorimetric micro-determination for thiols described by Saville (51). The stained product is formed according to a rather interesting reaction mechanism whose step is the already rather well-known conversion [transposition] of thiol with nitrous acid into nitrosylmercaptide.

$$R\text{--}SH + HONO \rightarrow R\text{—}S\text{—}NO + H_2O \qquad (28.17)$$

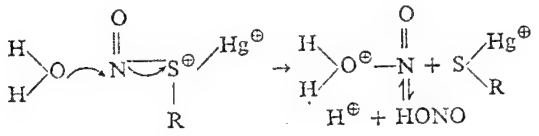
The excess nitrous acid is destroyed by amidosulfo acid in whose presence the nitrosylmercaptides are rather stable.

$$HONO + NH_4SO_3NH_2 \rightarrow NH_4HSO_4 + N_2 + H_2O \qquad (28.18)$$

After that, nitrosylmercaptide is hydrolyzed by mercury (II)-salt. This rapid hydrolysis can be explained in terms of the formation of a complex as a result of which the N-S bond becomes sensitive to the nucleophile attack of the water molecules and is split.

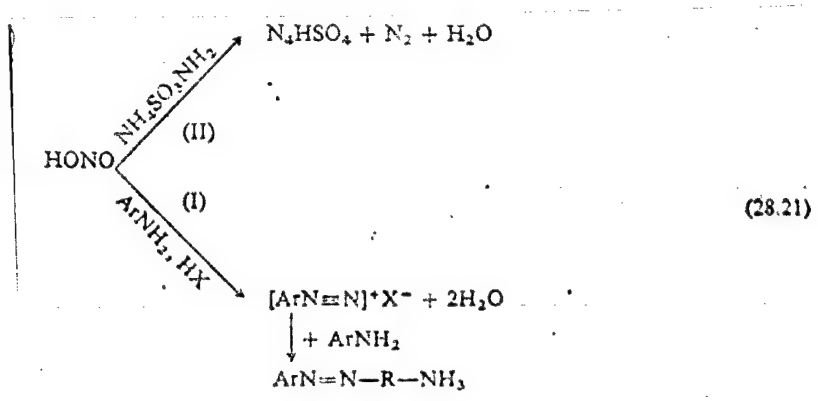


(28.19)



(28.20)

The nitrous acid released here will diazotize sulfanilamide (I); the diazonium salt is coupled with α-naphthylamine or another aromatic amine into an azo-dye. The competing reaction of nitrous acid with the amidosulfo acid present in the reaction mixture (II) essentially takes place more slowly and does not disturb the formation of the stain.



(28.21)

Reagents:

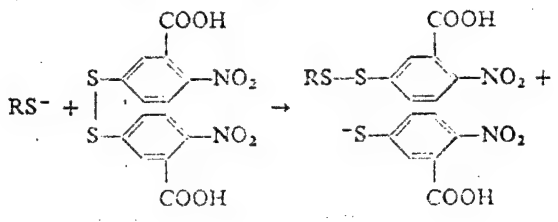
Solution I: Sodium nitrite, 0.03-percent, in water
Solution II: Amidosulfo acid, 2-percent, in water
Solution III: Mixture of:

1 part by volume 2-percent aqueous mercury (II)-chloride solution
4 parts by volume, 3-percent sulfanilamide solution in 2-percent hydrochloric acid
5 parts by volume, 0.1-percent solution of naphthylethylenediamindihydrochloride, 2-percent hydrochloric acid.

Operating Procedure

We heat 5 ml of the aqueous solution with 0.5 ml of 5 normal caustic soda in a ground-top reagent glass for 5 minutes in a water bath at 80° C. Then we add 1 ml of 5 normal sulfuric acid, we cool quickly, and we mix with 1 ml of solution I. One must avoid wetting the ground area with the solution as much as possible. After 1 minute we add 1 ml of solution II and we put the reagent glass vessel into the water bath after inserting the stopper and after shaking forcefully for 8 minutes. After we take it out, we once again shake forcefully, we slightly lift and turn the stopper so that the hot solution will thoroughly wet the ground surface along the glass and the stopper. Then we cool and we mix with 2 ml solution III. A red-violet stain, visible after several minutes, indicates thiolphosphoric acid esters up to a boundary concentration of about 0.1 µg/ml. If we use the same operating procedure, we can make the quantitative determination in that the stain obtained after 5 minutes is measured in the spectral photometer at 535 nm and we analyze with the help of a previously plotted calibration curve. For detection in alcoholic sample solutions, we mix 4 ml of the sample with 1 ml of water and 0.5 ml of 5-normal [standard] caustic soda and we heat for 15 minutes in a boiling water bath. Then we must add 4 ml of water and 1 ml of 5-normal sulfuric acid and, after cooling, we continue to work as described for the aqueous sample solution.

The thiol test with 5,5'-Dithio-bis-(2-nitrobenzoic acid) (52) used for the histochemical analysis of SH groups is of lesser sensitivity but very simple to perform. This water-soluble reagent (Elimann's reagent) reacts with thiols at pH 8, forming mixed disulfides and releasing the anion--which has an intensive yellow stain in the alkaline medium--of 5-thio-2-nitrobenzoic acid.

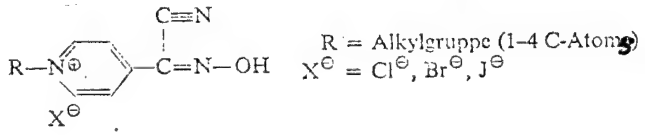


(28.22)

Bis-(p-Nitrophenyl)-disulfide (53) reacts in a similar manner but we must then work in acetonic-aqueous solution because of the low water solubility of this compound. Other stain tests of the hydrolytically split thiol can be performed with nitroprussid sodium, phosphorus-18-tungstic acid, 2,6-dichlorphenol-indophenol and triphenyltetrazolium chloride.

The detection of the tertiary amino nitrogen can be accomplished with the Dragendorff reagent or with phosphotungstic acid.

We get a specific staining reaction with organic phosphorus warfare agents of the type VX from oximes with the following general formula (328):



R = Alkylgruppe (1-4 C-Atome)
$X^{\ominus} = Cl^{\ominus}, Br^{\ominus}, J^{\ominus}$

(28.23)

Key: Alkylgruppe--Alkyl group.

In case of contact with V-warfare agents, these oxides reveal a color change from orange to blue, followed by red fluorescence in UV light. The compounds, which are also stable against the effect of light, are supposed to be particularly suitable for use in reference styluses, detection powders, and detection sprays.

Acetate-covered mixed polymerisates of chloral and dichloracetaldehyde are decomposed by various amino-nitrogen-containing compounds, including V agents (329).

This decomposition is made visible by dissolving the dye pinacyanol in the chloracetaldehyde formed. The detection system is particularly suitable for indicator vials.

28.1.1.6. Qualitative Differentiation of Organic Phosphorus Warfare Agents

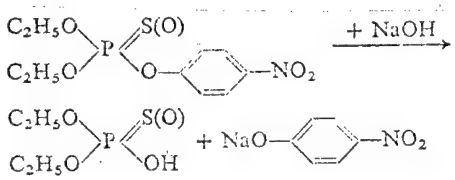
The differentiation of organic phosphorus warfare agents can be accomplished with the help of the above-described detection methods and the analysis of certain functional groups and elements. The following overview is designed to facilitate the analysis of the results obtained.

To begin with, the presence of an organic phosphorus warfare agent is determined through enzymatic detection with a general chemical analysis method, for example, the Schoenemann reaction.

We have:	In case of positive evidence of:
1. DFP	(a) Fluoride, after decomposition with sodium ethylate (b) Phosphate, after decomposition with strong caustic soda (c) O-isopropyl group with p-Dimethylaminobenzaldehyde-sulfuric acid
2. Tabun	(a) Cyanide, after decomposition with caustic soda (b) Dimethylamino group with Dragendorff reagent
3. Sarin	(a) Fluoride, after decomposition with sodium ethylate (b) O-isopropyl group with p-Dimethylaminobenzaldehyde sulfuric acid
4. Soman	(a) Fluoride, after decomposition with sodium ethylate (b) O-dimethylbutyl group with Vanillin-sulfuric acid
5. VX	(a) Dialkylamino group with Dragendorff reagent (b) Thiol sulfur as nitrosylmercaptide after alkaline cleavage (c) O-alkyl group

28.1.1.7. Detection of Paraoxon

The analysis methods described below are equally suited for the detection of the analogous thiono-compond, that is, parathion. The simplest form of detection is based on the acceleration of hydrolysis of this compound due to alkali.



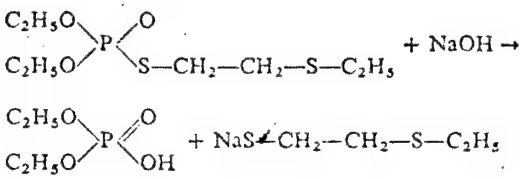
(28.24)

The intensive yellow color of the sodium salts of p-nitrophenol is then considered the analysis [detection] stain. The analysis is performed as follows: we mix 5 ml of the aqueous or alcoholic sample solution with 0.5 ml 20-percent lye and we heat for some time in a boiling water bath, possibly in the reflux. To increase the method's sensitivity, we can add Millon's [illegible in photostat] reagent. About 2-3 µg parathion will still be visible by virtue of a raspberry-red color (45).

The insecticides parathion, paraoxon, chlorthion, and EPN together with diphenylamin in a sulfuric acid solution give us an intensive blue coloration. The reaction mechanism corresponds to the known nitrate analysis.

28.1.1.8. Detection of Isosystox

A possibility of detecting isosystox is based on the ability of thioether-sulfur to form addition compounds with noble metal salts, especially with gold fluoride. These adducts are only slightly stained but are "developed" by chloramine or arylantidiazotate into heavily stained reaction products. This possibility, which now also applies to sulfure yperite, is used in the known systox detection vial by the Draeger Company. A simple performance of this test in the form of a spot test is described in connection with sulfur yperite. Like sulfur yperite, isosystox--if it is heated first of all in an alkaline solution and then acidified--produces a clouding effect with the reagent according to Mayer (HgJ_2-KJ); this clouding effect is visible up to a boundary concentration of 0.02 mg/ml. Other sensitive tests are possible if isosystox is hydrolyzed by heating with alkalis and if staining reactions are performed upon the mercaptan which is formed.



(28.25)

For example, the test can be performed in the following manner: 3 ml of the sample solution are mixed with 0.5 ml of 10-percent caustic soda and are heated to the boiling point. After cooling, we add three drops of a 10-percent nitroprussid-sodium solution. A red-violet stain, which is still recognizable at a boundary concentration of 0.1 mg/ml, indicates the presence of isosystox.

28.1.2. Quantitative Determination

28.1.2.1. Schoenemann Reaction

In the quantitative determination of organic phosphorus warfare agents with the help of the Schoenemann reaction--whose theory was described in detail in Section 28.1.1.1.--various modifications are possible. Some of them--in which differing reagents are used, will now be described in further detail.

Colorimetric Methods

(a) With o-Dianisidin (44)

Reagents:

Buffer solution, pH 11.5 (16 g potassium dihydrogenphosphate and 9 g potassium hydroxide are distilled in 1,000 ml and are dissolved in water free of any carbon dioxide; this solution is mixed with 1:1 purified acetone and is adjusted for a pH of 11.5 with diluted potash lye).

o-Dianisidindihydrochloride, 1.3-percent in water (the solution must be kept in the dark and must be freshly prepared each week!)

Hydrogen peroxide, 1-percent solution

Operating Procedure

We prepare a mixed reagent consisting of 23 parts of the buffer solution, one part o-dianisidindihydrochloride solution, and one part of hydrogen peroxide. This mixture is suitable for the determination of Sarin and Soman; if DFP or tetraethylpyrophosphate are to be determined, we add three parts of hydrogen peroxide solution. The mixed reagent can be kept up to 30 minutes.

Prior to the determination, we plot a calibration curve. For this purpose, we make a standard solution of the phosphorus-containing warfare agent in isopropanol. We use up to a maximum of 2 ml of this standard solution. The dilution series is to be so selected that, in the case of Sarin, those 2 ml--or aliquot (specimen) parts--will contain 0-300 µg Sarin. Aliquot parts in each case are supplemented from a microburet with isopropanol to 2 ml so that the reaction mixture will always contain the same quantity of alcohol.

We placed 20 ml of the reagent mixture into a 25-ml measuring flask and, drop by drop, while constantly mixing, we add the standard solution of the warfare agent within 60-90 sec. Then, if necessary, we supplement with isopropanol and we fill up to 25 ml with additional reagent mixture. At room temperature, the stain can then be measured in case of Sarin and Soman after 10 minutes, in case of DFP after 30 minutes, and in case of tetraethylpyrophosphate, it can be measured after 20 minutes in the colorimeter whose center of gravity is at 450 nm. As comparison solution we use a blank experiment in which, in place of the standard solution, we added 2 ml isopropanol. The extinctions determined for the various concentrations are then plotted in a calibration curve. The actual determination is then performed according to the same method and is analyzed according to the calibration curve.

The method has to be changed if we want to apply it to aminoalkylthiolphosphorus compounds. Satisfactory sensitivity is achieved if we either increase the hydrogen peroxide concentration and if we extend the time until we measure the stain or if the reaction mixture is briefly heated under reproducible conditions. We must of course devote special attention here to the blank experiment. The Schoenemann reaction cannot be used in determining quaternary compounds.

The method can, with minor changes, be adapted to the various tasks, such as the determination of organic phosphorus warfare agents in water or in the air (after absorption in a suitable solvent). The important thing is to make sure that the plotting of the calibration curve is in each case done with a standard solution which has the same composition as the sample solution. The share of the sample solution or the standard solution out of the reaction mixture can be increased to a maximum of 50 percent (12.5 ml), but the time until the attainment of the maximum stain is then extended and it must be determined in advance.

(b) With o-Tolidin (46)

Reagents

Buffer solution, pH 8.7 (1,000 ml of 0.1-molar potassium dihydrogenphosphate solution, 100 ml 1-molar caustic soda, and 1,000 ml of water are mixed and are adjusted for a pH value of 8.7 with 1-molar caustic soda, after which we dilute with water to 2 lit).

O-Tolidinhydrochloride, 1-percent, in water,

Sodium perborate, 1.25-percent, in water (must be prepared fresh daily!)

Operating Prodedure

To a mixed reagent, made immediately prior to use and consisting of 40 ml of purified acetone, 4 ml of buffer solution, 2 ml of o-tolidinhydrochloride solution, and 3 ml of sodium perborate solution, we add 10 ml of the aqueous sample solution. After 20 minutes, the stain is colorimetrized at 420 nm. For evaluation, we use a previously prepared calibration curve.

Fluorometric Method (47)

Reagents:

Solution I (in a mixture of 1,000 ml of water, 100 ml of acetone, and 100 ml of isopropanol, we dissolve 2 g of indol, 3.57 g of sodium bicarbonate, and 0.43 g sodium carbonate)

Solution II (3.5 ml 13.3-percent hydrogen peroxide are added to a mixture of 1,000 ml water and 250 ml isopropanol)

Operating Procedure

To 24 ml of a mixture of solution I and II (1:1), whose pH value should be at 10.6, we add 1 ml sample solution in isopropanol and we immediately determine the maximum fluorescence in the fluorescence photometer. Excitation is accomplished with UV light in the range of 350-400 nm and the maximum of the blue-green fluorescing is 500 nm. Analysis is performed with the help of a calibration curve.

28.1.2.2. Measurement-Analysis Determination of Organic Phosphorus Fluoride Compounds (Hydrolytic Method)

This determination method (48) can be used for checking on the purity of the mentioned phosphorus compounds as an easily handled macromethod. It is based on the difference of the hydrolysis speed of these compounds as compared to that of the impurities, such as phosphorylchlorides and difluorides. In order to distinguish between Sarin and the corresponding pyroester which is possible in the form of an impurity and which has similar hydrolysis properties, we can place the mixture on a water-saturated silicagel acid. The pyroester is retained while the Sarin is washed out with isopropylether and can then be titered.

The pyroester content then springs from the difference with respect to a previously performed overall determination or the pyroester can be separately determined in an aqueous extract. The time for alkaline hydrolysis after addition of caustic soda must be extended to 10 minutes as compared to 2-5 minutes for the Sarin determination.

Another simplified possibility for the separation of Sarin from pyroester is the reproducible distribution (80:20) between an organic solvent (isopropyl-ether) and an aqueous 3.6-percent sodium chloride solution. This separation is less accurate than column separation.

Operating Procedure

A volume of the phosphorus compound, corresponding to 0.13-0.18 g, is placed by means of a microflask pipet into a previously weighed Erlenmeyer flask and the exact weight is determined by means of re-weighing. Then we add 15 ml of water and 4 to 5 drops of a mixing indicator which consists of a solution of 0.05-percent methyl-red and 0.1 percent thymolphthaleine in methanol. More exact although more laborious is the weighing of the warfare agent in a glass ampoule which is then placed in the Erlenmeyer flask that is filled with water and indicator and which is crushed with a glass rod. After the dissolution of the phosphorus compound, we immediately titrate with 0.1-normal caustic soda up to the stain switch from red to yellow.

We note the consumption of caustic soda since it indicates the quantity of free acid and the quantities of acid from the rapidly hydrolyzing impurities. Immediately thereafter, we add additional caustic soda (about 2 ml per 0.01 g of the amount weighed in initially) until the solution takes on a blue color. This lye quantity is also recorded. After 2 or a maximum of 5 minutes, we retiter with 0.1-normal hydrochloric acid until the methyl-red changes color from yellow to yellow-pink. The Sarin content is then computed as follows:

$$\% \text{ Sarin} = \frac{7{,}005 \cdot (a \cdot F_{NaOH} - b \cdot F_{HCl}) \cdot 100}{\text{Einwaage [mg]}};$$

Key: Einwaage--Weighed portion.

a--Second consumption of 0.1 n NaOH (ml)
b--Consumption of 0.1 n HCl (ml)

Phosphorus compounds with poor water solubility, such as Soman, are dissolved in ethanol. Then we dilute with water until the alcohol content remains below 20 percent by volume. A higher alcohol content would reduce the hydrolysis speed of the fluorphosphonates.

28.1.2.3. Measurement-Analysis Determination of Organic Fluoride Phosphorus Compounds (Peroxide Method)

Using this method (50), it is possible to determine smaller quantities of fluorophosphonates with the help of the hydrolytic procedure. The method is based on the reaction of these phosphorus compounds with alkaline hydrogen peroxide solution and the iodometric determination of the excess peroxide.

$$H_2O_2 + 2\,HJ \rightarrow J_2 = 2\,H_2O \qquad (28.26)$$

Reagents:

Peroxide buffer solution, pH 10 (8.5-9 g sodium pyrophosphate peroxide, $Na_4P_2O{\cdot}2H_2O_2$, and 5.68 borax, $Na_2B_4O_7{\cdot}10H_2O$, are dissolved in 500 ml of water, they are adjusted to a pH of 10 with 10-percent caustic soda (about 12 ml), and they are filled up to 1 lit with water).

Operating Procedure

We weigh 0.18-0.24 g of the toxin in a 100-ml measurement flask in that the toxin is either pipetted in and the quantity is determined by weighing the flask or we use a glass ampoule which has been destroyed by means of a glass rod in the measurement flask previously filled with about 20 ml of 50-percent isopropanol. We fill up to the marker with 50--percent isopropanol. Into two 500-ml Erlenmeyer flasks with ground stopper, one of which is intended for the blank experiment, replace 50 ml, each, of the buffered peroxide solutions. Into one of the flasks we then pipette, while shaking constantly (important!), 20 ml of the sample solution and into the other flask we put 20 ml of 50-percent isopropanol and we allow both flasks to stand for 2-4 minutes while shaking occasionally. Then we add the following to each of the two solutions: 30 ml water, 10 ml 18-normal [standard] sulfuric acid, and 3 g potassium iodide, in this sequence; we mix and we allow the mixture to stand in the dark for 10 minutes. Then the separated iodine is retitered with 0.1-normal sodium thiosulfate solution. Shortly before the disappearance of the yellow iodine color, we add a little start solution as indicator and we titer up to decoloration. We find that 1 ml of the 0.1-normal thiosulfate solution consumed corresponds to 1/4,000 mole of fluorphosphonate. The Sarin content of the sample can then be calculated as follows:

$$\%\,\text{Sarin} = \frac{3{,}502 \cdot (a - b) \cdot F_{Na_2S_2O_3} \cdot 5 \cdot 100}{\text{Einwaage [mg]}};$$

Key: Einwaage--Weighed portion.
a = ml consumption of 0.1 n $Na_2S_2O_3$ for the blank test
b = ml consumption of 0.1 n $Na_2S_2O_3$ for the sample.

Phosphorylchlorides and difluorides do not disturb the determination because they hydrolyze in the aqueous-alcoholic sample solution already prior to the addition of peroxide. The values obtained with this method have an accuracy within 1 percent. A special determination of phosphorylchloride can be made after prior transposition with hydrofluoric acid to the corresponding phosphorylfluorides with the above method.

28.1.2.4. Fast Colorimetric Determination with 4-(p-Nitrobenzyl)-pyridine (p-NBP) (50)

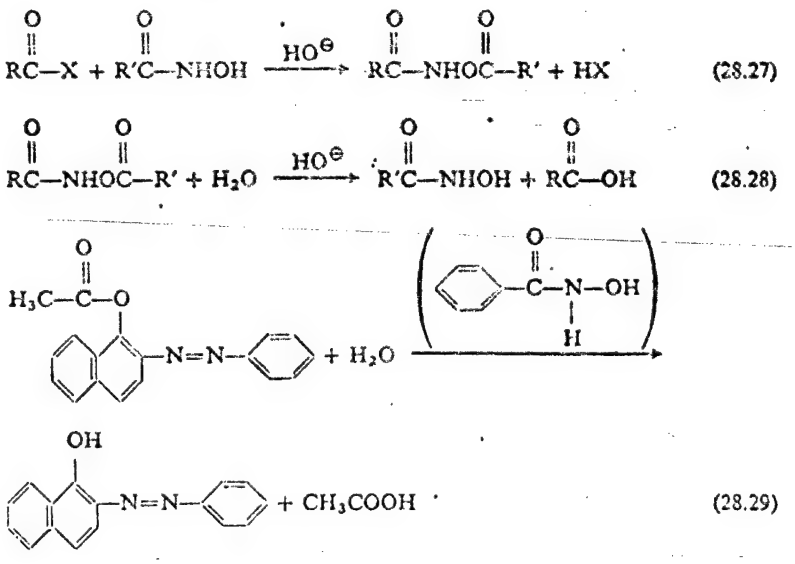
Reagents:

4-(p-nitrobenzyl)-pyridine, 2-percent in acetone; cyclohexylamine, 2-percent in acetone (the acetone needed is purified by means of boiling for 1 hour with 1 g $KMnO_4$ per liter and subsequent distillation.

We mix 0.05 ml of the solution of the toxin in acetone with 0.2 ml p-NBP solution and 0.2 ml cyclohexylamine solution and we heat for 3 minutes in the oil bath on the reflux. After cooling, we fill up with double-distilled ethylacetate to 3 ml and after 10 minutes we colorimetrize at 520 nm against a blank test. The analysis is accomplished by means of a calibration curve which was plotted 2-15 µg of the phosphorus compound.

28.1.2.5. Colorimetric Determination with Hydroxamic Acid

A very interesting detection and determination method for phosphoric acid esters (111) is based on the property of the hydroxamic acid ions to catalyze the hydrolysis of certain acylating compounds.



(28.27)

(28.28)

(28.29)

In this method, we allow the phosphoric acid ester to react with an excess of hydroxamic acid at pH 9 (see 28.1.1.4). The unused hydroxamic acid then catalyzes the hydrolysis of yellow 2-azobenzol-1-naphthylacetate, whereby red 2-azobenzol-1-naphthol is formed. After expiration of a certain interval of time, we stop the hydrolysis by adding acid. The stain intensity of the hydrolysis product is a measure of the original phosphoric acid ester quantity. The similarity to the enzymatic methods described in Chapter 29, based on the inhibition of cholinesterase is obvious but the chemical method is definitely less sensitive.

The optimum conditions for each phosphoric acid ester vary because they depend on the reaction speed of the ester with the hydroxamic acid ion and the hydroxide ion. The following operating instructions apply to Sarin:

Reagents:

$2 \cdot 10^{-3}$ molar solution of hexanehydroxamic acid in water
Buffer (0.001-molar solution of sodium tetraborate with 0.05 normal hydrochloric acid adjusted to a pH of 9).

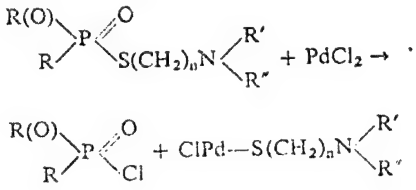
$2.5 \cdot 10^{-3}$ molar solution of 2-azobenzol-1-naphthylacetate in acetone
0.05-normal hydrochloric acid

Operating Procedure

We mix 4 ml of the aqueous sample solution with 1 ml of hydroxamic acid solution and 1 ml of buffer and we allow it to stand for 10 minutes at 25° C. After that we add 4 ml of the substrate solution, we mix, and we transpose after another 5 minutes with 0.5 ml hydrochloric acid. After 2 minutes, we determine the extinction in the spectral photometer at 540 nm. The analysis is performed with the help of a calibration curve. The smallest Sarin concentration that can be determined is 0.5 µg/ml.

28.1.2.6. Photometric Determination of Thiolphosphororganyls (V-Substances) (54)

The determination is based on the fast cleavage of the P-S bond by means of palladium (II)-chloride, with the formation of the corresponding phyosphoryl-chloride and a stable complex compound of palladium with thiol.



(28.30)

The palladium-thiol complex is determined through photometry in the UV range.

Reagents:

Palladiumchloride solution (0.05-molar solution of ammoniumchloropalladite in 0.3-molar hydrochloric acid)

Comparison solution (0.5 ml of the palladiumchloride solution are diluted 0.3-molar hydrochloric acid 50 ml)

Operating Procedure

The toxin is dissolved in water or in a solvent which can be mixed with water and which does not reveal any absorption in the wavelength range applied. Isopropanol is quite suitable here; 0.5 ml of the palladiumchloride solution are mixed in a 50-ml measuring flask with a certain volume of the toxin solution; then we fill up to the marker with 0.3-molar hydrochloric acid. The absorption is measured at 250 nm or 310 nm against the comparison solution.

The analysis is performed according to a calibration curve which was plotted with a standard solution of the corresponding thiol compound. The time until the attainment of maximum absorption depends on the type of thiol compound and must be determined in each case.

This determination method can be used for all thiol compounds which form water-soluble palladium complexes. We can determine concentrations of about 2-200 µg/ml.

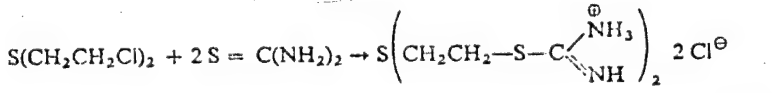
28.2. Halogenated Thioether (Sulfur Yperites)

In accordance with the significance of Bis-(2-chlorethyl)-thioether during World War I, numerous analysis methods were worked out at that time. Because of the development of more sensitive staining reactions, they are today only of minor interest and we can therefore dispense with a detailed description in favor of the modern methods.

28.2.1. Qualitative Analysis Method

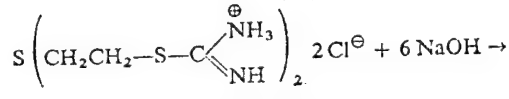
28.2.1.1. Reaction with Thiourea and Nickel Salt

In this reaction, described by Harley-Mason (55), the chlorine atoms of sulfur yperite are replaced with thiol groups and the dithiol is established as red complex compound with nickel.



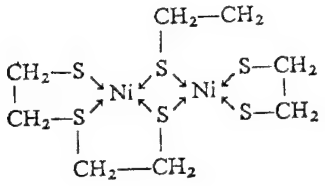
(28.31)

This product is split in an alkaline solution into bis-[ethanthiol-(2)]-thioether.



(28.32)

Dithiol then, with ammonia-alkaline nickel salt solution, gives us a red complex with the following structure:



(28.33)

The performance of the staining reaction was worked out by Matousek and Tomecek (42).

Reagents:

Thiourea, 5-percent, in ethanol
Nickel sulfate (we dissolve 5 g in 50 ml water and we fill up to 100 ml with 20-percent ammonia solution)
Caustic soda, 30-percent
Acetic acid, 50-percent

Operating Procedure

To 1 ml of the alcoholic sample solution we add 1 ml thiourea solution and we heat in a water bath to the boiling point for 2-3 minutes. Then we add 0.5 ml caustic soda and we once again quickly heat to the boiling point. After cooling, we neutralize or we slightly acidify with acetic acid. After adding 0.5 ml nickel sulfate solution, we get a red stain in the presence of yperite; that stain is shaken out after dilution of the sample solution with 1-2 ml of water, with 2-3 ml of chloroform or with dichloroethane. In place of the nickel sulfate solution we can also add 0.5-percent nitroprussid sodium solution with which we get a violet color in the alkaline medium.

Boundary Concentration: 0.04 mg sulfur or nitrogen yperite per milliliter

Irregularities: In the presence of higher concentrations of acetone bromide, chloropicrin, chloracetophenon, methyl- and ethyldichlorarsis, similar stains are obtained after some time.

28.2.1.2. Reaction with Alkaline Thymolphthaleine Solution

This staining reaction, which is equally useful in the detection of sulfur and nitrogen yperites, is based on the following reaction mechanism (see Formula 28.34, above).

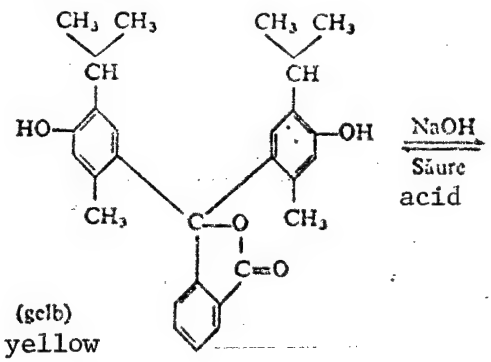
The makeup of this dye, which is stable in acid solution, was confirmed by Matousek and associates (42) through ultrared-spectroscopic investigations.

Reagent:

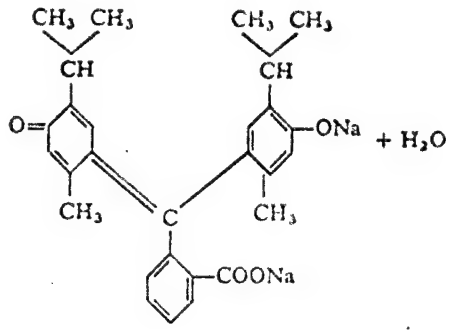
0.4 g thymolphthaleine are dissolved in 60 ml of pure ethanol and 20 ml of 0.6-percent potash lye are added to the solution. The solution should assume a deep blue color here.

Operating Procedure

To 2 ml alcoholic or aqueous sample solution, we add the same volume of reagent and we heat the mixture for 20 minutes to 80° C in a water bath. We must make sure that the mixture will retain a blue color during heating, otherwise we would have to add several drops of diluted potash lye until the blue color is restored. After cooling, we add one or two drops of acetic acid, whereupon the blue stain will disappear and the solution, in the presence of sulfur or nitrogen yperite, will show a yellow to orange-red stain.

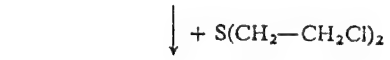


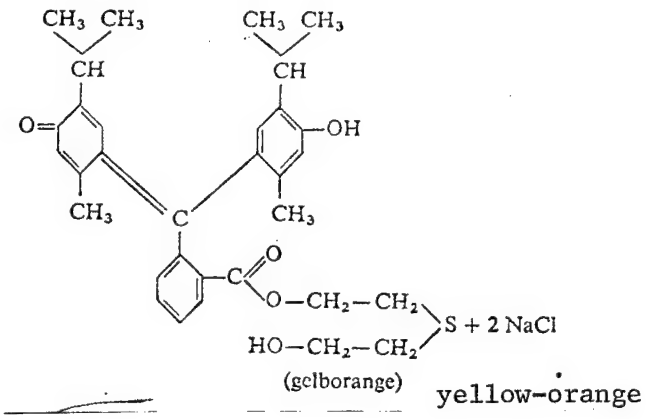
(gelb)
yellow



(28.34)

(blau)
blue





(gelborange) yellow-orange

A slight coloration becomes clearly recognizable if we shake out with 1 ml toluene or benzene. When we use an alcoholic sample solution, we must add a little water before shaking out. After adding 1:1 diluted hydrochloric acid to the toluene layer, the coloration of the same will change to red. If we are dealing with nitrogen yperite, the reaction product with thymolphthalein will contain a basic nitrogen atom and the red coloration will move into the hydrochloric acid layer due to the formation of a water-soluble salt. The reaction can also be used for the photometric determination of yperites in that we measure the extinction at 450 nm and analyze with the help of a calibration curve.

Boundary concentration: 0.05 µg sulfur or nitrogen yperite per milliliter.

Disturbances: Halogen-containing aliphatic compounds, such as dichloroethane, acetone bromide, chloropicrin and others produce identical stains.

28.2.1.3. Reaction with Grignard Reagent

The detection reagent described by Grignard (56) contains complex sodium copper iodide which, when combined with copper sulfite and an excess of sodium iodide is formed in aqueous solution:

$$CuSO_4 + 2\,NaJ \rightarrow CuJ_2 + Na_2SO_4 \qquad (28.35)$$

$$2\,CuJ_2 \rightarrow Cu_2J_2 + J_2 \qquad (28.36)$$

$$Cu_2J_2 + 2\,NaJ \rightarrow Na_2Cu_2J_4 \qquad (28.37)$$

During the reaction with sulfur yperite, an insoluble compound of bis-(2-iodine-ethyl)-thioether is formed with copper iodide:

$$Na_2CuJ_4 + S(CH_2{-}CH_2Cl)_2 \rightarrow S(CH_2{-}CH_2J)_2CuJ_2 + 2\,NaCl \qquad (28.38)$$

The addition of gum arabic will stabilize the developing clouding.

Reagents:

20 g sodium iodide are dissolved in 50 ml of water and we add 1 ml 7.5-percent copper sulfate solution. After adding 2 ml of a solution of 35 g gum arabic in 100 ml water, we fill up with water to 200 ml and we filter. The reagent must be kept in a flask made of brown glass.

Operating Procedure

We can suction air to be tested through an absorber flask filled with the reagent or the reagent can be added to the aqueous sample solution. The presence of sulfur yperite is indicated by a yellow clouding.

Boundary concentration: 0.05 mg/ml.

Irregularities: The analysis is disturbed by arsines which produce identical clouding. Marbot (57) described a drop test to be performed with the reagents used in the Grignard test.

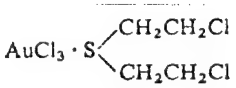
Operating Procedure

A drop of the alcoholic sample solution is placed on filter paper. After the evaporation of the solvent, we place, in the same spot, a drop of 5-percent alcoholic sodium iodide solution and we dab the latter along the edge with a drop of 10-percent copper sulfate solution. Upon observation in the light of a UV lamp, we note a yellow-green fluorescence in the presence of sulfur yperite along the contact zone of the drop.

Boundary concentration: 0.1 mg/ml.

28.2.1.4. Reaction with Gold Chloride

In the reaction, used for the first time by Schroeter (58) and Obermiller (59) in detecting sulfur yperite, an insoluble addition compound is formed.



(28.39)

Identical deposit compounds are formed with many heavy metal salts but gold and palladium salts are best suited for this analysis. The reaction is well suited for drop tests on paper and is used in combination with sodium thiosulfate, chloramine-T, or arylantidiazotates which develop a coloration with the addition compound; they are frequently used as reagent systems for indicator vials.

When making the determination in an aqueous solution, the yellow clouding which has developed can be stabilized by adding a little solution of a heteropoly-acid (phosphorus molybdenum acid, phosphorus tungstic acid, silico-tungstic acid) probably due to molecule enlargement.

Drop test: A drop of the sample solution is placed on filter paper and is mixed with a drop of 5-percent gold chloride solution and then with a drop of 1-percent chloramine-T solution. A red-brown coloration of the spot indicates sulfur yperite.

Boundary concentration: 0.1 mg/ml.

28.2.1.5. Miscellaneous Detection Possibilities

The protection or determination method described for nitrogen yperate with 4-(p-nitrobenzyl)-pyridine and 8-oxyquinoline (see section 28.3.2.1. and 28.3.2.2.) are, when slightly modified, also suitable for the detection of sulfur yperite. Other testing methods which, however, regarding their practical use, reveal disadvantages due to low sensitivity or specificity and awkward handling, are possible with the following reagents:

(a) Potassium permanganate in a 0.003-percent solution is discolored by sulfur yperite; the boundary concentration is about 0.15 mg/ml.

(b) β-naphthol, in an alcoholic, heavily alkaline solution, will cause clouding. The boundary concentration is at 0.06 mg/ml.

(c) Potassium mercury iodide (10 g potassium iodide and 14 g mercury iodide in 70 ml of water) will produce a yellowish-white precipitate.

$$K_2[HgJ_4] + S(CH_2CH_2Cl)_2 \rightarrow S(CH_2CH_2J)_2 \cdot HgJ_2 + 2KCl$$

The boundary concentration is 0.05 mg/ml.

(d) Selenic acid (1 g SeO_2 in 100 ml of 1:1 diluted sulfuric acid) will produce an orange suspension or yellow precipitate with sulfur yperite and with arsine when heated to 85°.

(e) 2,6-dichlorphenol-indophenol. The blue redox indicator is reduced to a colorless shape at a pH of 7.0. The recording boundary in 0.01 mg.

(f) Copper (II) salts will form an additional compound. The detection limit is 0.1 mg.

(g) Nessler reagent will produce a yellowish-white precipitate.

(h) Iodate and starch in an acid solution will cause a blue stain.

28.2.1.6. Identification by Preparation of Derivatives

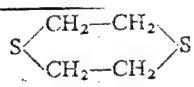
If larger quantities of sample material, for example, from ammunition discovered in various places, should be available, then reliable identification is possible by means of conversion into derivatives with a defined melting point.

(a) Sulfimine

During the reduction of sulfur yperite with an excess of aqueous solution of chloramine-T, white crystals of sulfimins will be separated after about an hour [CH_3-C_6H_4-SO_2-N = $S(CH_2CH_2Cl)_2$], whose melting point is 144.6° C.

(b) Diethylenesulfide

When we add sulfur yperite to a 20-percent solution of sodium sulfide, diethylenesulfide (dithion) is precipitated with a melting point of 111-112° C.



(28.40)

(c) Sulfoxide

When we add sulfur yperite drop by drop to the concentrated nitric acid (D.1,40), we get a bright-green liquid from which sulfoxide is precipitated as a white

precipitate in case of dilution with water. After recrystallization from 60-percent ethanol, we get colorless scales with a melting point of 110° C. Sulfoxide can also be made by dissolving sulfur yperite in glacial acetic acid and adding 30-percent hydrogen peroxide while cooling.

28.2.2. Quantitative Determination

28.2.2.1. Photometric Determination with Iodoplatinate

Chugayev found that organic sulfides react with iodoplatinate according to two possible reactions, with iodine being released in each case.

$$PtJ_6^{2\ominus} + 4R_2S \rightarrow Pt(R_2S)_4^{2\oplus} + 4J^{\ominus} + J_2 \qquad (28.41)$$

$$PtJ_6^{2\ominus} + 2R_2S \rightarrow Pt(R_2S)_2J_2 + 2J^{\ominus} + J_2 \qquad (28.42)$$

This fact can be used for the determination of bis-(2-ethyl chloride)-thioether if the iodine formed is detected or determined by means of the blue color of the adsorption compound obtained with starch. A series of determination methods with visual or photometric analysis is based on this principle. Compared to other methods, these methods offer the advantage that they are subjected to disturbance by polysulfides only to a minor degree.

Reagents (61)

Iodoplatinate (to 1 ml of a 5-percent solution of ammonium chloroplatinate, we add 3.5 ml of a freshly made 5-percent sodium iodide solution which must be free of iodine and we dilute the mixture with distilled water to 180 ml).

Starch solution (1 g soluble starch is ground up with a little water and is placed in 100 ml of boiling water; the solution must in each case be freshly prepared).

Acetic acid (50 ml of glacial acetic acid is diluted with distilled water to 1,000 ml).

Operating Procedure

The aqueous sample solution must be 5-percent acetic acid. To 10 ml of this sample solution we add 2 ml of iodoplatinate solution. After mixing, we add 2 ml of starch solution. We note the time.

After 4-5 minutes, we colorimetrize at 650-700 nm against a comparison sample which was set up with 10 ml 5-percent acetic acid. The analysis is performed according to a calibration curve which is plotted with sulfur yperite solution in 5-percent acetic acid containing concentrations between 0.005 and 0.05 mg/ml.

28.2.2.2. Measurement Analysis Determination with Bromide-Bromate Solution

Thioethers are stoichiometrically oxidized into sulfoxide in diluted aqueous solution.

$$R_2S + Br_2 + H_2O \rightarrow R_2SO + 2HBr \qquad (28.43)$$

Bis-(2-ethyl chloride)-thioether can be titered with an adjusted bromide solution whereby the terminal point is established with methyl-red. In place of the unstable bromide solution, we use bromide produced in situ electrolytically or through bromide-bromate in an acid solution. The use of the method is confined to pure sulfur yperite because polysulfides and thiodiglycol likewise react with bromine which interfere with the determination. To eliminate the error caused by the indicator's bromine consumption, it is a good idea to mix with an excess of bromide-bromine and to determine it iodometrically (63).

Operating Procedure

In a 250-ml Erlenmeyer flask with ground stopper we weigh 0.1-0.3 g of the warfare agent and we dissolve in 40 ml of glacial acetic acid. After adding 5 ml of water and 3 ml of concentrated hydrochloric acid, we slowly titer with 0.1-normal bromide-bromate solution (11.906 g KBr and 2.784 g $KBrO_3$ in 1 lit of solution) until we get a yellow color. Then we add 5 ml of a 10-percent potassium iodide solution. We allow the mixture to stand in the dark for 5 minutes and we titer the separated iodine with 0.1-normal [standard] sodium thiosulfate solution, adding a little starch solution as indicator. The computation is performed according to the following formula:

$$\% \text{ sulfur yperite} = \frac{7.952 \cdot (a-b) \cdot 100}{E};$$

a--Consumption of 0.1-normal bromide-bromate solution in milliliters
b--Consumption of 0.1-normal thiosulfate solution in milliliters
E--Weighed portion of warfare agent in milligrams

28.2.2.3. Measurement Analysis Microdetermination

In addition to the formation of sulfimine (see Section 28.2.1.6.a.) we can also get sulfoxide or sulfone in case of a reaction of bis-(2-ethylchoride)-thioether with chloramine-T in an aqueous solution.

$$CH_3—C_6H_4—SO_2NCl^{\ominus} + R_2S + H_2O \rightarrow CH_3—C_6H_4—SO_2NH_2 + R_2SO + Cl^{\ominus} \qquad (28.44)$$

$$2CH_3—C_6H_4—SO_2NCl^{\ominus} + R_2S + 2H_2O \rightarrow 2CH_3—C_6H_4—SO_2NH_2 + R_2SO_2 + 2Cl^{\ominus} \qquad (28.45)$$

The product to be formed depends on the acidity of the solution and on the type of acid. In an acetic-acid (up to 50 percent) and sulfuric-acid solution (up to 3-normal), we consume 1 mol chloramine and 90-100 percent of the reactor product are sulfoxide. In 2-normal hydrochloric acid on the other hand we consume 2 mol and sulfone is formed. In this case, the oxidation is brought about by the chlorine which is formed in an intermediate fashion. A method for yperite determination is based on the addition of chloramine-T in acetic-acid

solution and colorametric determination of the excess with o-tolidin. A method worked out by Kinsey and Grant (62) achieves higher sensitivity. According to those authors, in the reaction of bis-(2-ethylchloride)-thioether with dichloramine-T in a water-free medium, in a complicated chlorination, 5 mol dichloramine-T are consumed per mol of yperite. The consumption of dichloramine depends on the solvent. Cyclohexane is suitable; the addition of cyclohexanol proves to be sensitizing for the catylatic chlorination of the yperite.

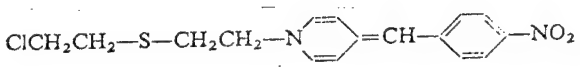
Operating Procedure

The warfare agent is dissolved in a mixture 80 percent cyclohexane and 20 percent purified kerosine (boiling point: 175-280° C) or poisoned water is taken out with this mixture. We then mix 1 ml of the solution in a 50 ml Erlenmeyer flask with ground stopper with 1 ml of a 20-percent solution of dichloramine-T in carbontetrachloride and we place it for 20 minutes in a water bath adjusted for 27±0.1° C after that we add 4 drops of saturated potassium iodide solution and 4 drops of glacial acetic acid and, after thorough soaking, the titer is 0.01-standard sodium thiosulfate solution until decoloration. We must not work in sunlight. This method is suitable for the determination of 5-200 µg of sulfur yperite. Computation is impossible because the dichloramine consumption is subject to certain fluctuations that depend on the purity of the solvent. One must therefore plot a standard titration curve with solutions of known yperite content and one must analyze according to it.

For the determination of smaller quantities of sulfur yperite (0.2-5 µg) we add, to 1 ml of the warfare agent solution in cyclohexane, 1 ml of a 5-percent solution of cyclohexanol in kerosine. The mixture is adjusted for 27±0.1° C in a water bath and, after the addition of 1 ml 0.1-percent solution of dichloramine-T, in carbon tetrachloride, it is kept at this temperature for exactly 20 minutes. After that, we add potassium iodide and acetic acid, as above, and the separated iodine is titered with 0.01-standard thiosulfate solution. In plotting the calibration curve, we must consider the thiosulfate used up during a blank test. We find that 1 ml of 0.01-standard thiosulfate solution corresponds to about 5 µg sulfur pyrite. In both methods, the exact maintenance of the temperature and the time is critical.

28.2.2.4. Colorimetric Determination of 1,2-bis-(2-Ethylchloride)-Ethane (Sesquiyperite) in Mixtures with Bis-(2-Ethylchloride)-Thioether (Yperite) (60)

This method is suitable for the determination of sesquiyperite in mixtures with bis-(2-ethylchloride)-thioether after both yperites have first been determined colorimetrically with 4-(p-nitrobenzyl)-pyridine. For this purpose we heat the warfare agent mixture dissolved in ethyl alcohol for 15 minutes at 70° C with 4-(p-nitrobenzyl)-pyridine and we add piperidine as base (see Section 28.3.2.2.). We get a violet-colored compound.



(28.46)

28.2.2.5. Other Methods to Determine Bis-(2-chloroethyl)-thioether

/Text/ The colorimetric determination of sulphayperite is also possible with the methods indicated in the qualitative detection with phthalein and thiourea-nickel salt. The method described for the detection and determination of nitrogen yperite with 8-oxyquinoline is also applicable to sulfayperite and also the generally applicable method with 4-(p-nitrobenzyl)-pyridine. Other methods described are the following:

a) Titration with adjusted sodium hypochlorite solution using methyl red or neutral red as indicators.

b) Precipitation as double salt with copper (I)-chloride and iodometric determination of the excess copper salt.

c) Nephelometric determination of the suspension of metal selenium obtained under heating with selenic acid.

d) Reaction with the sulfhydryl groups of cysteine, urease, papain or denatured ovalbumin and determination of the remaining SH groups. The method is suitable to determine all the agents attacking the sulfhydryl groups, such as bromacetone, chlorpicrin, iodic and bromic acetic acid ester and sulfa yperite.

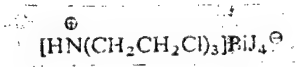
28.3. Halogenated Aliphatic Tertiary Amines (Nitrogen Yperites)

28.3.1. Method of Qualitative Detection

Besides the application of the method described in the qualitative detection of sulfa yperite with alkaline phthalein solution and thiourea nickel salt, a number of precipitation reagents for tertiary amines are particularly suitable for the detection of nitrogen yperite. Most alkaloids and the N,N-dialkylaminoalkylphosphates, phosphonates and thiophosphonates give similar precipitations and disturb these detections.

28.3.1.1 Precipitation with Potassium Bismuth Iodide (Reagent According to Dragendorff/ Kraut)

The orange to red precipitate has the following composition



$$[HN(CH_2CH_2Cl)_3]BiJ_4^{\ominus}$$

Reagents:

Dragendorff reagent (produced by pouring together a solution of 8 g basic bismuth nitrate in 20 ml nitric acid (D.1.18) and a solution of 27.2 g potassium iodide in 40 ml water, after being left standing for a long time (1 to 2 days) is poured away from the potassium nitrate deposited and the solution is filled up to 100 ml).

Procedure:

5 ml of the aqueous specimen solution are treated with 0.5 ml hydrochloric acid (diluted one to one) and with 1 ml Dragendorff reagent. An orange-red deposit indicates nitrogen yperites.

Limiting Concentration:

0.01 mg/ml are still detectable.

Interferences:

Higher concentrations of sulfa yperite (more than 1 mg) give a similar reaction and naturally all the chemical agents (VX) and psychological poisons (BZ) with tertiary nitrogen atom as well as many alkaloids are detected with this reagent.

28.3.1.2. Precipitation with Phosphorotungsticacid

To avoid interferences by lewisite, dichloromethylarsine and dichloroethylarsine, adamsite and bromobenzylcyanide, the detection must be carried out in hydrochloric acid.

Reagents:

Phosophorotungsticacid, 5 percent solution in water.

Procedure:

5 ml of the aqueous specimen solution are treated with 0.5 ml hydrochloric acid and 1 ml phosphorotungsticacid. A cloudiness or gelatinous deposit indicates nitrogen yperites.

Limiting Concentration:

0.01 mg/ml may be detected.

28.3.1.3. Other Precipitation Reagents

a) With silicotungsticacid a precipitate is obtained of composition 12WO3.SiO2. /N(CH2CH2CL)3/4 which allows also the gravimetric determination of nitrogen yperites.

b) Reinecke salt (NH4/Cr(NH3)2(SCN(4/) and other complex chromium salts give with nitrogen yperites pink precipitates, which allow the detection of 0.005 mg/ml.

c) With Nessler reagents in cold conditions a yellow deposit is obtained and a brown precipitate under heat. It is possible to detect about 0.2 mg/ml.

28.3.1.4. Detection by Production of Derivatives

In the simplest case the melting points of the hydrochlorides recrystallized from acetone can be used for identification. The hydrochloride of the methyl-bis-(2-chloroethyl)-amine melts at 110 degrees C and that of the tri-(2-chloroethyl)-amine at 130 to 131 degrees C. Another possibility is the precipitation of picrates. The aqueous specimen solution is treated with saturated aqueous picrate acid solution. With larger amounts the yellow picrates depositing can be recrystallized from ethanol diluted with some water. The picrate of the bis-(2-chloroethyl) amine melt at 133 degrees C, that of the tri-(2-chloroethyl)-amine at 135 degrees C. The picrolonate of the tri-(2-chloroethyl)-amine obtained by precipitation with picrolonic acid has the same melting point.

28.3.2. Quantitative Determination

28.3.2.1. Colorimetric Determination with 8-Oxyquinoline (Oxine) [64]

The basis for this very specific method is the formation of a colored product not previously defined in the reaction between nitrogen and sulfayperites with 8-oxyquinoline in the alkaline medium.

Reagents:

8-Oxyquinoline, 5 percent solution in 95 percent ethanol.

Sodium carbonate, 10 percent solution in water.

Acetic acid, 5 percent.

Procedure:

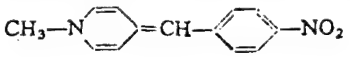
2.5 ml of the aqueous specimen solution are mixed with 2.5 ml 95 percent ethanol and slightly acidulated with the addition of a few drops of acetic acid. Thereafter 0.5 ml of the 8-oxyquinoline solution are added, agitated and after adding 0.5 ml sodium carbonate solution agitated once again for 30 seconds. After 90 minutes standing at room temperature the colorimetric study is carried out at 486 nm against a controlled experiment. The evaluation takes place with a calibration curve which is recorded with 0.01 to 1 mg agent per milliliter. In the determination of the sulfayperite heating is carriedout for 90 minutes to 45 degrees C and the measurement at 500 nm.

The qualitative detection can be carried out on the basis of the bright orange color (the blank test is yellowish-green) occurring in the presence of nitrogen yperites.

28.3.2.2. Colorimetric Determination with 4-(p-nitrobenzyl)-pyridine [66]

This method of detection and determination applicable under manifold aspects for the alkylizing compounds is based on an observation by Koenig who had obtained in the reaction of methyliodide with 4-(p-nitrobenzyl)-pyridine in the alkaline medium a

blue dye, to which the following structure was attributed:



During the Second World War in the US the general applicability of this dire reaction was established and various qualitative detections were developed for chemical agents such as sulfayperite, diphenylchloroarsine and others. Epstein /65/ continued these studies and established quantitative determinations for ethylene imines and various alkalyzing compounds, including many chemical warfare agents. By comparing the intensity of the dye it was possible to determine the activity of the different compounds or their functional groups as compared with 4-(p-nitrobenzyl) pyridine:

$$RCH_2CH_2X > RCH_2CHX_2 > RCH_2CX_3$$

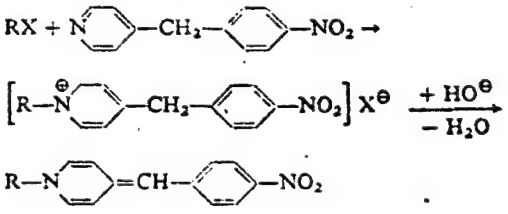
$$ArCH_2X > ArX$$

$$ROCH_2X > ROCH_2CH_2X$$

$$COCH_2X > COX$$

(X = Halogen)

In the halogens the sequence iodine bromine chlorine was established. The reaction takes place through the formation of a quarternary pyridinium salt, which in the presence of organic or inorganic bases (piperidine, triethylamine, alkaline carbonate amine, alkaline carbonates and hydroxides) is converted into the colored compound with the loss of a proton.



Besides other alkylizing compounds the following warfare agents give this color reaction:

Alkylfluorophosphonates (Sarin and Soman) /40,50/.

Sulfayperite /65/.

Secondary and tertiary nitrogen yperites /66/.

Arsenic warfare agents (lewisite, methyl, ethyl and phenyldichloroarsine and diphenyldichloroarsine /65/.

Methylfluoroacetate /65/.

Chloroacetophenone /67/.

The conditions of the reaction for the optimum production of the color are different for each compound. The limiting concentrations are for some compounds for example sulfa yperite at a few micrograms per milliliter, for others like alkylfluorophosphonates, the sensitivity is lower.

Reagents:

4-(p-nitrobenzyl)-pyridine (melting point 70 to 71 degrees C, 5 percent solution in acetone).

Acetate buffer, 0.1 normal, pH 4.62.

Sodium hydroxide, 0.25 normal.

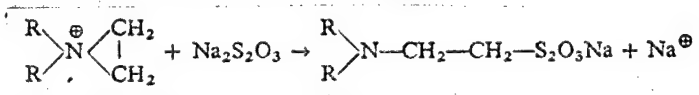
Procedure:

3 ml of the aqueous toxic solution are pipetted into a test tube with a ground stopper and the latter is placed in a boiling water bath for 20 minutes after adding 1 ml acetate buffer and 0.4 ml of 4-(p-nitrobenzyl)-pyridine solution. Then it is cooled in an ice bath and 2 ml acetone, 5 ml ethylacetate and 1.5 ml 0.25 normal sodium hydroxide are added in this sequence.

The glass is agitated about 20 times, and after pouring the content into a centrifuge glass, centrifuging is carried out for 2 minutes. The corresponding amount of the upper layer is pipetted into a dish and colorimetry is carried out at 540 nm against a reference specimen to which water was added. Because of the instability of the color formed, the operations from the addition of the sodium hydroxide until the measurements had to be carried out within 3 to 5 minutes and protected from light. The evaluation takes place according to a calibration curve which was plotted in the same way with 0.5 to 10 µg nitrogen yperite.

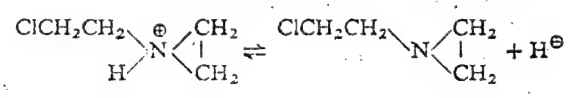
28.3.2.3. Other Methods of Determination

a) The hydrolysis of nitrogen yperites with tertiary nitrogen takes place through the fast formation of an ethyleneimonium ion. The speed of formation increases with the increase of the hydroxide ion concentration; at pH 6 the conversion is centrally completed after 5 minutes. Von Golumbic, Fruton and Bergmann established that the ethyleneimonium ion (also called aziridine ion in the Anglo-Saxon literature) can be detected by a conversion with thiosulfate.

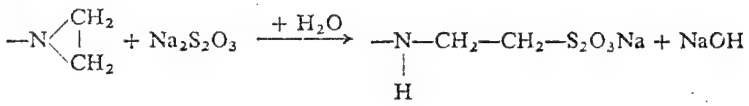


But it was found that the addition of an excess of thiosulfate and the retitration with iodine solution did not give any qualitative values. The cause may be sought in the occurrence of secondary reactions, for example the hydrolysis of the ethylene imonium ion or the dimerization. $HgCl_2 + 2HCN \rightarrow Hg(CN)_2 + 2HCl$

The hydrolysis of nitrogen yperites with secondary aminonitrogen takes place in a different way. According to the pH value an equilibrium is adjusted between the ethyleneimonium ion and ethyleneimine.



From pH 3 to 5 the speed of decomposition of the secondary nitrogen yperite increases in a manner similar to that of the tertiary compounds, later to fall to zero at about pH 8, from which the stability of the ethylene amine occurring at this pH value against a nucleophile attack is apparent. Allen and Seaman induced the reaction of ethylene amine with thiosulfate.

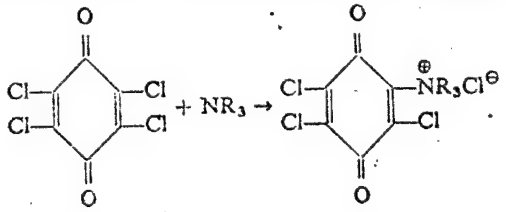


These authors established that the ethyleneimine compound can be determined quantitatively by reaction with a high excess in thiosulfate at pH 4 and determining the acid consumption of the reaction. In a solution with bicarbonate as buffer the decomposition of the secondary nitrogen yperites led to oxazolidone derivatives, so that it was impossible to establish any thiosulfate consumption.

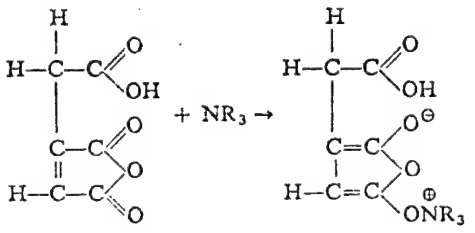
b) A possibility to determine the tris-(2-chloroethyl)-amine consists in the precipitation with potassium bismuth iodide, the dissolution of the precipitate with the addition of 8-oxyquinoline and the bromometric determination after dissolving the bismuthoxyquinolate in hydrochloric acid. Amounts up to 0.1 mg can be determined with high precision.

c) Methyl-bis-(2-chloroethyl)-amine can be determined by conversion with p-phenylphenol in alkaline solution and measurement of the fluorescence of the reaction product.

d) Two colorimetric methods of determination for tertiary amines and their salts and for quarternary amines were described by Sass and his colleagues [68]. The known detection methods for amines with chloranil in which red colors were obtained with primary amines, violet with secondary amines and emerald green with tertiary amines, was modified. By photometry of the dye at 610 nm free tertiary amines can be determined even mixed with salts up to a limited concentration of 50 µg/ml.



e) To determine the salts of tertiary amines, quarternary amines and free tertiary amines the red color occurring in the reaction of amines with cis-aconiticanhydride in acetic anhydride was used.



The limiting concentration of the determination is at 3 µg/ml; the color is photometrized at 500 nm. By applying both methods it is possible to determine free tertiary amines besides their salts or the quarternary form.

f) Through the determination of nitrogen yperites with alkaline thymolphthalein reagent as well as with thiourea and formation of thioronium salts data are obtained in the description of the similar method for sulfayperites (compare section 28.2.1.1. and 28.2.1.2.).

28.4. Chemical Warfare Agents and Poisons Containing Arsenic

28.4.1. Qualitative Methods of Detection

To analyze the warfare agents containing arsenic it should be noted that besides the specific analytical determination of the individual agents the toxic decomposition products retaining to a great extent their arsenic content must be noted. This applies in particular in the examination of drinking water and foodstuffs.

28.4.1.1. General Methods of Detection

Reduction to Hydrogen Arsenide.

Hydrogen in the "status nascendi" reduces primary and secondary aliphatic arsines such as dimethyl and ethyldichloroarsine, 2-chlorovinyldichloroarsine (α Lewisite) and bis-(2-chlorovinyl)-chloroarsine (β-lewisite), to hydrogen arsinide. For tris-(2-chlorovinyl)-arsine (γ-lewisite) and the aromatic arsines such as phenyldichloroarsine, diphenylchloroarsine (Clark I), diphenylcyanoarsine (Clark II) and diphenylaminechloroarsine (adamsite) this direct reduction is impossible. These compounds must previously be mineralized by means of a method indicated in the quantitative determination of chemical warfare agents containing arsenic (compare section 28.4.2.1). The arsenic trioxide obtained is then reduced. The detection of the hydrogenarsenide produced can be carried out with:

a) Silver nitrate [69].
A lemon yellow spot of AsAg3.AgNO3 is formed on a paper impregnated with concentrated silver nitrate solution. When treated with water the spot is colored black by separation of metal silver.

b) Mercury (II)-bromide [70].
Reagent paper impregnated with this substance gives a yellow to brown color which deepens to red-brown by treatment with potassiumiodide solution

$$3\,HgBr_2 + AsH_3 \rightarrow As(HgBr)_3 + 3\,HBr$$

$$As(HgBr)_3 + AsH_3 \rightarrow As_2Hg_3 + 3\,HBr$$

c) Goldchloride [71].
On a paper soaked with 1 percent gold chloride solution by reduction to metal gold a blue to bluish-red spot is formed. This detection has the maximum sensitivity.

$$2\,AuCl_3 + AsH_3 + 3\,H_2O \rightarrow 2\,Au + 6\,HCl + H_3As_3O_3$$

For detection with silver nitrate the following procedure applies:

Reagents:

Reagent paper (impregnated immediately before use) with a 20 percent solution of silver nitrate).
Metal zinc, granulated and free from arsenic.
Sulfuric acid, 20 percent.

Procedure:

A few milliliters of the specimen solution or attack solution acidulated with sulfuric acid are poured into a small test tube and a small crystal of copper sulfate and two zinc granules are added. After fixing in the upper part of the tube a plug of lead acetate cotton, the reagent paper is placed on the openings. A yellow spot formed after some time on the paper and which becomes black immediately when treated with water indicates warfare agents containing arsenic.

Detection with Hydrogen Sulfide

Through the formation of the corresponding arsenic sulfides the precipitates are separated. 2 to 3 ml of the aqueous specimen solution are treated until precipitation with a freshly prepared solution of hydrogen sulfide acidulated with hydrochloric acid. In the presence of aromatic arsines the procedure is carried out with alcoholic specimen solution and saturated alcoholic solution of hydrogen sulfide.

If necessary ice water must be used for cooling in the separation of sulfides. It is advantageous to apply the so-called solid hydrogen sulfide; these are compounds such as thioacetamide or ammonium thiocarbanate which separates hydrogen sulfide in acid solution. The following arsenic sulfides are precipitated individually:

a) Lewisite gives a white deposit of ClCH=CH-As=S. The limiting concentration is 0.02 to 0.05 mg/ml.

b) Dimethylchloroarsine gives a yellowish deposit of CH3-As=S with a melting point of 110 degrees C.

c) Diethylchloroarsine gives an emulsion of yellowish oil. The limiting concentration is 0.02 to 0.05 mg/ml.

d) Diphenylchloroarsine gives a white deposit of C6H5-As=S with a melting point of 152 degrees C.

e) Diphenylchloro- and cyanoarsine gives a white deposits of (C6H5)2As2S with a melting point of 67 degrees C.

Detection with Mercury (I)-Nitrate

If a few drops of a weak nitric acid 5 percent aqueous solution of mercury (I)-nitrate are added to the aqueous specimen solution, deposits are obtained for:

a) α-lewisite (white deposit of calomil, Hg_2Cl_2 which becomes gray within 12 hours; the limiting concentration is 1 mg/ml).

b) Dimethylchloroarsine (gray deposit of metal mercury; with limiting concentration of 1 mg/ml).

c) Diethylchloroarsine (white deposit which quickly becomes gray; the limiting concentration is 2 mg/ml).

d) Diphenylchloroarsine (white deposit).

Diphenylchloro- and cyanoarsine form a white deposit only after some time.

Detection with Hypophosphorus Acid

Except for adamsite or warfare agents containing arsenic give with hypophosphorus acid characteristic deposits. According to Bougault the reagent solution is prepared as follows:

10 g sodium hyposulfite is dissolved under heating in 10 ml water and concentrated hydrochloric acid is added up to 100 ml. After decanting of the deposited sodium chloride we add per 10 ml solution 1 to 2 drops 0.1 normal iodine-potassium iodide solution.

Procedure:

1 to 2 ml of the aqueous specimen solution are treated with 5 ml reagent. The formation of a deposit is observed and heating applied if the latter does not occur for about 15 to 30 minutes in the water bath. In the presence of chemical agents containing arsenic we obtain with lewisite a white, and later yellow deposit, for methyl- and ethylarsinic-dichloride a white to yellowish-brown deposit and for diphenylchloroarsine a white to yellowish-green deposit and for diphenylchloro- and cyanoarsine after heating a white turbidity.

Sulfur yperite in high concentration gives an emulsion; under heating the solution becomes clear by the agglomeration of the drops.

Other General Methods of Detection

a) Primary aliphatic arsine, such as -lewisite, dimethyl- and diethylchloroarsine reduced osmiumtetroxide to the black osmiumdioxide.

$$OsO_4 + 2RAsCl_2 + 4H_2O \rightarrow OsO_2 + 2RAsO(OH)_2 + 4HCl$$

Silica gel impregnated with 1 percent osmiumtetroxide solution can be used for indicator tubes.

b) The reaction of primary aliphatic arsines with tin (II)-chloride in concentrated acid is also suitable for indicator tubes, in accordance with the Bettendorf detection. A yellow ring is formed on suitably impregnated silica gel when the air containing lewisite is sucked through.

c) A silica gel impregnated with molybdic acid and zinc sulfate is colored blue when air containing alkyldichloroarsines are sucked through because of the reduction of molybdic acid. This reaction is very sensitive.

d) The products obtained in the oxidation of diphenylarsines with iodine solution give with acetic acid urinylnitrate solution deposits, with green color for adamsite.

e) The chemical agents containing arsenic can be detected with high sensitivity except adamsite, with the iodine copper reagent according to Grignard.

Detection through the Production of Derivatives

The hydrolysis and oxidation products and the above-mentioned sulfides are suitable for identification purposes. A survey is given below of the melting points of these compounds:

Hydrolysis of	gives	melting point
methyldichloroarsine	CH3AsO	95 degrees C
α-lewisite	ClCH=CHAsO	143 degrees C
β-lewisite	/(ClCH-CH)2As/2O	62 to 63 degrees C
diphenylchloro- and cyanoarsine	/(C6H5)2As2/)	92.5 to 93.5 degrees C
adamsite	/HN(C6H4)2As/2O	350 degrees C

Oxidation of	gives	melting point
dimethylchloroarsine	CH3AsO(OH)2	159 degrees C
diethylchloroarsine	C2H5AsO(OH)2	99 to 100 degrees C
α-lewisite	ClCH=CHAsO(OH)2	130 degrees C
β-lewisite	(ClCH=CH)2AsO(OH)	120 to 122 degrees C
diphenylchloro and cyanoarsine	(C6H5)2AsO(OH)	175 degrees C

28.4.1.2. Special Methods of Detection

Detection of Lewisite

a) The detection of the acetylene formed in the decomposition of α-lewisite with strong alkali lye with the Ilosvay reagent [72] as copper acetylide is very sensitive and specific for lewisite.

$$ClCH{=}CHAsCl_2 + 6NaOH \rightarrow 2CH{\equiv}CH + Na_3AsO_3 + 3H_2O + 3NaCl$$

$$CH{\equiv}CH + 2Cu^{\oplus} \rightarrow CuC{\equiv}CCu + 2H^{\oplus}$$

Procedure:

2 ml of the aqueous or 1 ml of the alcoholic specimen solution, mixed with 1 ml water are treated with 0.5 ml 30 percent alkali lye. After sealing the test tube mixing is accomplished by slight tilting. After 5 minutes the solution is acidulated slightly with dilute acetic acid, while the neutralization heat is removed quickly by cooling with water. After adding 1 ml reagent a red color appearing immediately or after a short time with high concentrations a red deposit indicates the presence of α-lewisite in the specimen.

Limits of detection:

0.005 mg lewisite per milliliter can be detected.

Interferences:

The detection is specific.

An addition of 2 percent gelatin solution keeps the copper acetylide in colloidal form in the solution so that by comparison with standard solutions a quantitative evaluation can be carried out. A modified reagent [73] contains 0.2 g copper carbonate, 12 g arsenictrioxide, 12 g sodiumhydroxide and 1 ml piperidine in 100 ml water and gives a bright red color for detection. Moreover the precipitation with silver nitrate is possible as silver acetylide.

b) Silica gel which is impregnated with a solution of ergosterin[1] in chloroform gives when agitated with lewisite vapors a violet color and with higher concentrations it becomes deep green. Sulfur yperite produces with longer effect of higher concentration only a week coloring; hydrochloric acid vapors give with the reagent a deep rust brown color.

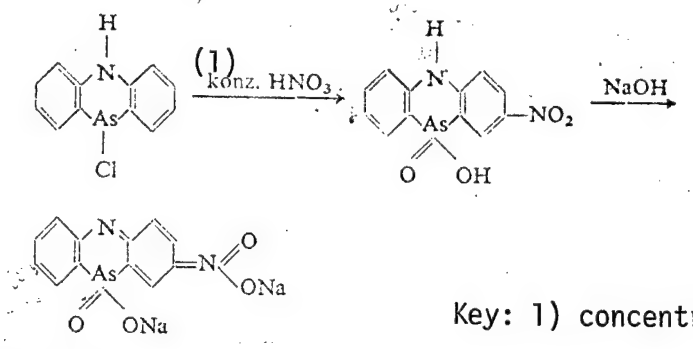
Detection of Diphenylcyanoarsine

The presence of diphenylcyanoarsine in a specimen can be detected by heating with alcoholic potassium lye and the detection of the cyano group identified by one of the usual methods.

Detection of Adamsite (Diphenylaminochloroarsine)

a) A simple possibility of detection is offered by the intense red coloring which is found when pouring over a few crystals of the specimen substance (or the residue after the evaporation of an extract) of concentrated sulfuric acid. The nature of the colored product is not known. With bromobenzylcyanide a brownish-red color is obtained and with bromobenzyl heated a red color.

b) The detection in the form of intensely red sodium salt of the nitration product is highly sensitive and specific for adamsite.



Key: 1) concentrated

[1] $C_{26}H_{44}O$ provitamin; in the irradiation with ultraviolet light it is converted to vitamin D2.

Reagents:

Concentrated nitric acid.

Soda lye, 30 percent.

Procedure:

A drop of nitric acid is added to a few crystals of the chemical agent or the residue which was obtained after evaporation of 5 to 10 ml of a solvent extract and evaporation carried out on the water until dry. After cooling a drop of soda lye is added to the residue. A cherry red color indicates the presence of adamsite.

Detection Limits:

About 0.5 to 1 µg adamsite are detectable.

c) The detection which is based on the formation of diphenylamine under heating with hydriodic acid is more complicated to accomplish.

$$HN(C_6H_4)_2AsCl + 2HJ \rightarrow (C_6H_5)_2NH + AsClJ_2$$

The diphenylamine is distilled with water vapor and according to the principle of the known nitrate detection can be identified after adding nitrate and concentrated sulfuric acid by a blue color. The blue coloring which is obtained with the addition of concentrated sulfuric acid to which some nitric acid was added to adamsite is also based on the diphenylamine reaction.

d) A reagent which consists of equal volumes of 10 percent silver nitrate solution and glacial acetic acid, indicates specifically the presence of adamsite. By heating on the water bath the stable greenish-yellow color is formed which is visible even with 0.02 mg adamsite.

Differentiation of the Chemical Agents Containing Arsenic

Methyl-, ethyl- and phenyldichloroarsine as well as diphenylchlorarsine for which no special detection processes are known are differentiated through the characteristic course of general detection processes with hydrogen sulfide, mercury (I) nitrate and the Bougault reagent (compare Table 28.2) after which arsenic was established by means of the reduction detection process (compare section 28.4.1.1.).

28.4.2. Quantitative Determination

28.4.2.1. Reduction to Hydrogenarsenide

The general principle of this method has already been discussed in section 28.4.1.1. in the description of the qualitative detection. Before reduction in many cases the mineralization of the specimen substance is needed. This applies in the presence of aromatic arsines which cannot be reduced directly and in the determination of chemical agents containing arsenic in foodstuff specimens, especially in fats. The mineralization is possible by a series of methods, of which the following one was selected as suitable for the examination of foodstuffs [117].

About 100 g of the material to be studied are crushed and mixed thoroughly. 2 to 3 g are placed in a Kjeldahl container and treated with 5 ml 30 percent halogen peroxide (perhydrol). After about 10 minutes 10 ml concentrated sulfuric acid are added in drops and the vessel is carefully heated with a small flame. When the content of the vessel begins to get brown, heating is stopped, cooling is carried out and after adding a few milliliters of hydrogen peroxide the heating is continued until the appearance of sulfuric acid vapors. This is repeated until the liquid no longer becomes brown with boiling up to 15 minutes. In case of fats the attack solution may react for several hours. Thereafter 0.5 g hydrizine sulfate is added without allowing any to remain suspended in the neck of the vessel. After repeated half an hour heating then the contents of the vessel cooled again in which arsenic occurs in the trivalent form is used for the determination. In a similar way a smaller amount of the isolated warfare agent or the alcoholic extract from foodstuffs and fats can be treated. The omission of the reduction of the arsenic occurring after the digesion in the pentavalent form to the trivalent form by means of hydrizine sulfate causes the reduction to hydrogen arsenide to slow down and increases the time for the evaluation of the determination to more than double.

Reagents:

Mercury (II)-bromide paper (filter paper is soaked for about 1 hour with 5 percent alcoholic solution of $HgBr_2$ and dried in the air, the paper is kept protected from light in the exsiccator on phosphorus pentoxide).

Granulated zinc free from arsenic (it is placed before use for 1 to 2 minutes in a 0.05 percent copper sulfate solution and rinsed with water).

Sulfuric acid 20 percent free from arsenic.

Lead acetate cotton (surgical cotton is soaked with 5 percent solution of lead acetate and dried).

Procedure:

10 ml of the attack solution previously filled up to a desired volume are poured in the vessel of a Gutzeit apparatus (see Figure 28.1). After adding 20 ml sulfuric acid water is filled up to 50 ml. After the lower tube of the attachment was coated with a loose plug of lead acetate cotton and the upper tube with a band of mercury bromide paper, 5 g zinc are added to the vessel, the cover is set up quickly and kept for 1 hour in the darkness. The evaluation takes place generally with comparison with a series of papers which were colored brown by reduction of arsenic trioxide solutions of definite concentrations. Such papers can be used for some time as reference scale when kept in the darkness. The length of the colored area is a measure of the amount of arsenic when papers of certain dimensions are used. For 2 mm width the following values apply [70]:

2	5	9	11	13	14,5	16	18	20	22	mm
0,02	0,05	0,1	0,15	0,2	0,25	0,30	0,35	0,40	0,50	mg Arsch (1)

Key: 1) arsenic

In other variants of the attachment for the Gutzeit apparatus the reagent paper is stretched in a calibrated opening. The round spot is compared with the self-produced reference papers or with a printed color scale. The accomplishment of a control test is advisable for checking the reagents (zinc, sulfuric acid) for freedom from arsenic in every case. With this method up to about 1 µg arsenic can be determined.

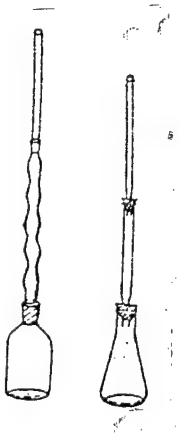


Figure 28.1: Instruments for determining arsenic according to Gutzeit.

A very sensitive and easily reproduced method is the colorimetric determination of the hydrogen arsenide produced with silver diethyldithiocarbamate [11].

Reagent:

0.5 percent solution of silver diethyldithiocarbamate in freshly distilled pyridine. The reagent solution when kept in the darkness and cooled can be preserved for several months.

The production of the silver salt is carried out by the following method: 2/25 g diethyldithiocarbamicacid or the calculated amount of sodium salt are dissolved in 100 ml water. By adding in parts a silver nitrate solution (1.7 g AgNO3 in 100 ml water) the lemon yellow silver salt is precipitated, filtered away and after thorough washing in water dried in the vacuum exsiccator. Thereafter the salt is kept in the darkness.

Procedure:

The production of hydrogen arsenide takes place in the usual manner. The vessel for the production is connected through the transfer tube through an absorption vessel containing a measured volume (5 ml) of the reagent solution. After 30 minutes the extinction of the red color formed is measured at 540 nm. The evaluation takes place on the basis of a calibration curve plotted with arsenictrioxide. The detection limit of the method is at 0.05 µg arsenic.

The method is disturbed by antimonyhydride which also gives a red color with the reagent. Hydrogensulfide gives a black color. By inserting a plug of lead acetate cotton in the transfer tube or the neck of the production vessel this disturbance can be prevented by the decomposition of the hydrogen sulfide as lead sulfide.

Table 28.2 : Differentiation of Chemical Warfare Agents Containing Arsenic

No.	Warfare Agent	Hypophosphoric Acid	Hydrogen Sulfide	Mercury (I) Nitrate	Ilosvay Reagent	Concentrated Sulfuric Acid
1	lewisite	white, later yellow-brown deposit	white deposit	white deposit, slowly becoming gray	red	
2	methyl dichloroarsine	white to yellow-brown deposit	yellowish deposit	gray deposit		
3	ethyl dichloroarsine	white to yellow-brown deposit	yellowish oil	white deposit, quickly becoming gray		
4	phenyl dichloroarsine	white to yellow-brown deposit	white deposit	white deposit		
5	diphenylchloroarsine	after heating white turbidity	(white turbidity)	(white deposit)		
6	diphenylcyanoarsine	after heating white turbidity	(white turbidity)	(white deposit)		
7	adamsite					red

The reactions in parentheses are only visible after some time.

28.4.2.2. General Iodometric Determination of Chemical Agents Containing Arsenic

In this method applicable in the determination of all the poisons containing arsenic the substance is mineralized and the arsenic acid formed is titrated iodometrically.

012 to 0.3 g of the poison are weighed in a Kjeldahl vessel and after adding 10 ml concentrated sulfuric acid and 1 ml fuming nitric acid it is heated for 1 hour until boiled. After cooling 50 ml water are added, evaporation is carried out and the treatment repeated as described above. After cooling once again 10 ml water, 2 g potassium iodide and if there is a deposit more water is added successively until the dissolution. After standing for 30 minutes in the dark the iodine separated is titrated with 0.1 normal sodium thiosulfate solution.

1 ml 0.1 normal sodium thionate solution corresponds to

8.043 mg methyldichloroarsine
8.744 mg ethyldichloroarsine
11.15 mg phenyldichloroarsine

13.228 mg diphenylchloroarsine
12.756 mg diphenylcyanoarsine
13.878 mg diphenylaminochloroarsine.

28.4.2.3. Iodometric Determination of Methyl-, Ethyl- and Phenyldichloroarsine

The determination is based on the oxidation of the product of hydrolysis of the poisons with iodine into arsonic acid

$$RAsCl_2 + 4NaOH \rightarrow RAs(ONa)_2 + 2NaCl + 2H_2O$$

$$RAsO + J_2 + 2H_2O \rightarrow RAsO(OH)_2 + 2HJ$$

Procedure:

1 g of the poison is weighed in a 100 ml standard vessel and agitated with 20 ml water and 15 ml 10 percent soda lye until complete dissolution. After filling to 100 ml each time 25 ml is pipetted into Erlenmeyer flasks, treated with 30 ml water and 1 to 2 drops of phenolphthalein indicator and neutralized with dilute sulfuric acid until the pink color disappears.

After cooling 50 ml saturated sodium bicarbonate solutions and 1 ml star solution are added. Thereafter the titration is carried out with 0.1 normal iodine solution until the color becomes blue.

1 ml 0.1 normal iodine solution corresponds to:

8.043 mg methyldichloroarsine
8.744 mg ethyldichloroarsine
11.15 mg phenyldichloroarsine.

28.4.2.4. Iodometric Determination of Diphenylchloroarsines

The principle of the determination is also the hydrolysis and the oxidation with iodine into diphenylarsonic acid.

$$(C_6H_5)_2AsCl + J_2 + 2H_2O \rightarrow (C_6H_5)_2AsO(OH) + 2HJ + HCl$$

Procedure:

0.2 to 0.4 g of the chemical agent are weighed in an Erlenmeyer flask and dissolved in 10 to 15 ml benzol or chloroform. After adding 20 ml saturated aqueous sodium bicarbonate solution the titration is carried out with 0.1 normal iodine solution with violent agitation until the color of the solvent turns violet. 1 ml of the 0.1 normal iodine solution corresponds to 13.228 g diphenylchloroarsine.

28.4.2.5. Colorometric Determination of Diphenylaminochloroarsine (Adamsite)[74]

The basis of this method is the color reaction described in section 28.4.1.2.

Reagents:

Mixture of 10 percent concentration nitric acid and 90 percent glacial acetic acid.

Procedure:

0.2 to 1 ml of the solution of chemical agent are treated with 0.5 ml reagent and 10 ml 20 percent soda lye. After filling with water up to 20 ml the violet color is subject to colorimetry at 530 nm. The evaluation is carried out according to a calibration curve which was plotted with 1 to 8 μg adamsite.

1 to 5 μg adamsite can be determined by colorimetric evaluation of the orange-yellow suspension formed with the addition of sulfuric acid to the acetone solution with the addition of an emulsifier (polyalkylglycolether).

28.5. Hydrocyanic Acid and Cyanichalides

In view of their importance as poisons and their manifold application in chemical industry and electroplating techniques, a large number of methods have been established todetect and determine prussic acid, its salts and compounds. Many of the methods described for hydrocyanic acid and cyanides are applicable for the analytical detection of cyanic halides.

28.5.1. Methods of Qualitative Detection for Hydrocyanic Acid and Cyanides

28.5.1.1. Berlin Blue Reaction

The formation of Berlin Blue is a specific detection for cyanide anions.

$$Fe^{2\oplus} + 2CN^{\ominus} \rightarrow Fe(CN)_2$$

$$Fe(CN)_2 + 4CN^{\ominus} \rightarrow [Fe(CN)_6]^{4\ominus}$$

$$Fe(CN)_6^{4\ominus} + 4Fe^{3\oplus} \rightarrow Fe_4[Fe(CN)_6]$$

Procedure:

Some milliliters of the specimen solution are treated until the weakly alkaline reaction with soda lye. 2 to 3 drops of 1 percent iron(II)-sulfate solution are added, heated and treated with 2 to 3 drops of 10 percent iron(III)-chloride solution. The greenish-blue to blue color formed after acidulation with dilute hydrochloric acid or a blue deposit shows the presence of cyanide. The limiting concentration is 0.02 mg/ml.

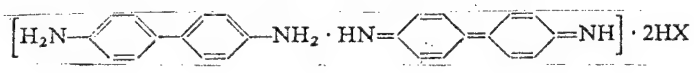
28.5.1.2. Detection of Copper Acetate/Benzidine Acetate [83]

This detection applied very often is based on the increase of the oxidation potential of copper(II) salts as compared with benzidine by the formation of insoluble copper(I) cyanide.

$$Cu(Ac)_2 + 2HCN \rightarrow Cu(CN)_2 + 2HAc$$

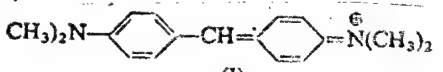
$$2Cu(CN)_2 + H_2O \rightarrow Cu_2(CN)_2 + 2HCN + {}^1/_2O_2$$

Benzidine is oxidized into a merichinoid blue compound.



The detection system is used predominantly as paper test (compare seciont 32.2.3.) or in test tubes. The advantage of the use of o-tolidine is that reagent solution and the

color produced are more stable; on the other hand it is oxidized more easily. The same advantages are obtained by substituting benzidine by tetramethyldiaminodiphenylmethane (methane base or tetra base). A blue chinoid compound is formed or a carbonium cation (I or II). Since benzidine has a highly cancerogenic effect, the methane base should be preferred.



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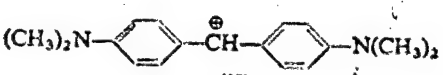


(II)

The drawback of this detection process is the disturbance by a large number of oxidation agents.

28.5.1.3. Detection with Sodiumpicrate

Sodium picrate gives with hydrocyanic acid a sodium salt of isopurpuric acid. The detection is carried out in the presence of alkaly as paper test of in solution. A reddish-brown to orange-red color of the yellow solution is obtained. The limiting concentration is at 0.3 mg/ml. Reducing substances disturbs the detection.

28.5.1.4. Detection by Formation of Nondissociated Heavy Metal Cyanides

In the reaction of hydrocyanic acid with mercury(II)-chloride an equivalent amount of hydrochloric acid and nondissociated mercury cyanide are formed.

The hydrochloric acid is detected by indicators such as Congo Red, bromothymol blue or methyl orange. This detection process in which silver nitrate as well as copper and palladium salts can be used is suitable for testing papers and tubes.

28.5.1.5. Demasking of Metal Chelates

1. Palladiumdimethylglyoxime

In this detection process dimethylglyoxime (D) is demasked from an alkaline solution of the sodium-palladiumdimethylglyoxime (PdD2) through the effect of cyanide. With the addition of nickel chloride solution containing ammonium salts, a red nickel dimethylglyoxime precipitates.

$$[PdD_2]^{2\oplus} + 4CN^{\ominus} \rightarrow [Pd(CN)_4]^{2\ominus} + 2D^{2\ominus}$$

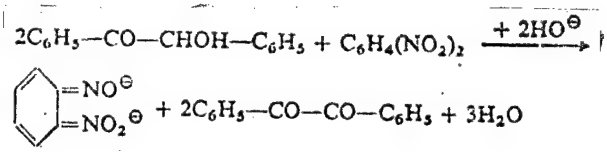
The limiting concentration is at 1 µg cyanide/ml.

2. Copper(II)-oxinate

The 8-oxyquinoline demasked in the reaction of cyanide ions with copper(II)-oxinate forms with the added aluminum salt the fluorescent aluminum oxinate. It is possible to detect 2.5 µg cyanide.

28.5.1.6. Detection by the Catalytical Acceleration of the Benzoine Condensation [84]

The known benzoine condensation is catalyzed by traces of cyanide. By using a sensitive color reaction on the benzoine formed with o-dinitrobenzol this fact can be used to detect microquantities of cyanide.



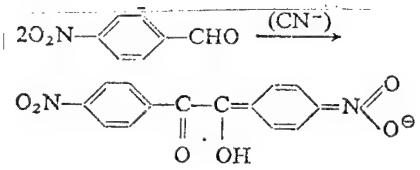
Reagents:

Benzaldehyde (0.5 ml are treated with 0.5 ml 25 percent soda lye and 4 ml ethanol, the reagent must be colorless and is to be freshly produced each time).

o-dinitrobenzol, 5 percent in benzol.

Procedure:

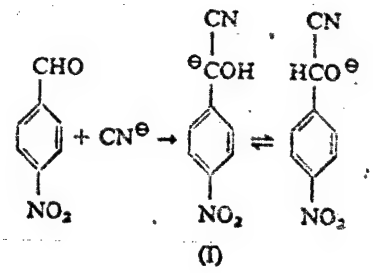
In a microtest tube 1 drop of the specimen solution is treated with 1 to 2 drops of benzaldehyde solution and heated for 2 to 5 minutes on the water bath. A drop of dinitrobenzol solution is added with further heating. According to the concentration of cyanide after 1 to 3 minutes a more or less intense violet color is produced. It must be compared each time with the controlled test. The detection limit is 0.01 µg. In the presence of sulfides and compounds which act in alkaline solutions as hydrogen donors, the detection cannot be used. Similarly through the catalytical effect of cyanide ions from p-nitrobenzalhyde in the presence of alkali a colored azyloine condensation product is formed [85].



A further increased sensitivity was achieved by Guilbault and Kramer [86] by the simultaneous use of p-nitrobenzaldehyde and o-dinitrobenzol. The cyanohydrin reduces o-dinitrobenzol to the blue dianion of the o-nitrophenylhydroxylamine. Since the cyanide ion is formed again in this reaction and can react once again, the detection achieves a very high sensitivity.

Reagents:

A. p-nitrobenzaldehyde, 0.2 molar in glycolmonomethylether
B. o-dinitrobenzol, 0.2 molar in glycolmonomethylether.
C. soda lye, 0.5 molar.



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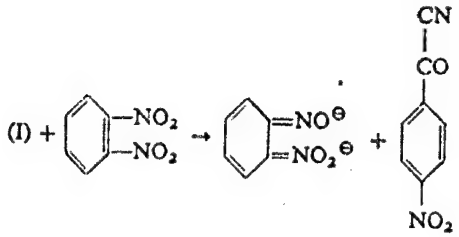
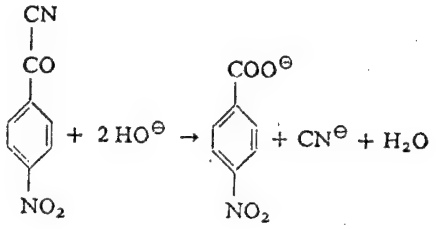


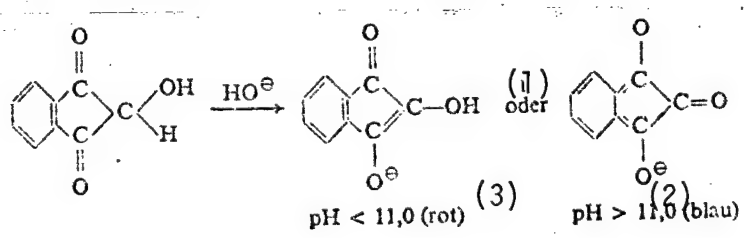


Procedure:

1 ml specimen solution is treated with 0.5 ml A and B and 0.2 ml C. A purple color indicates the presence of cyanide. The detection limit is at 0.01 µg cyanide.

By measuring the extinction at 560 nm the method can be used for the quantitative determination of cyanide and chemical agents which produce cyanide in the hydrolysis.

Similarly ninhydrine reacts with cyanide ions. The cyanohydrin formed in the intermediate stage gives according to a not totally clarified reaction course 2-oxy-1,3-diketohydrinden while the cyanide ions are formed once again. The 2-oxy-1,3-diketohydrinden forms according to the pH value two anions of different color.



Key: 1) or; 2) blue; 3) red

Reagents:

A. Potassium sodium carbonate, 5 percent in water
B. Ninhydrine, 5 percent in ethanol.

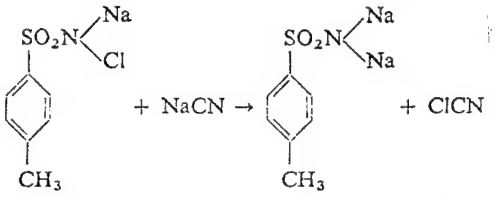
Procedure:

1 ml of the specimen solution is treated with 1 drop each of A and B and mixed by swiveling. According to the cyanide concentration a red color appears immediately or after short standing. The limiting concentration is 0.1 µg/ml.

When a drop is added of 0.5 percent solution of o-dinitrobenzol in ethanol to the reaction mixture just as in the previously described detection systems a stable violet color is obtained and 0.05 µg cyanide/ml can be detected.

28.5.1.7. Detection by Formation of Cyanohalide

But one of the most important possibilities of detection for prussic acid is the conversion by chloramines or bromine water into cyanogen chloride or bromide and the application of the detection system described in section 28.5.2.1. for cyanogen halides. When using bromine water after 1 to 2 minutes the excess is destroyed by adding arsenic acid or phenol solution.

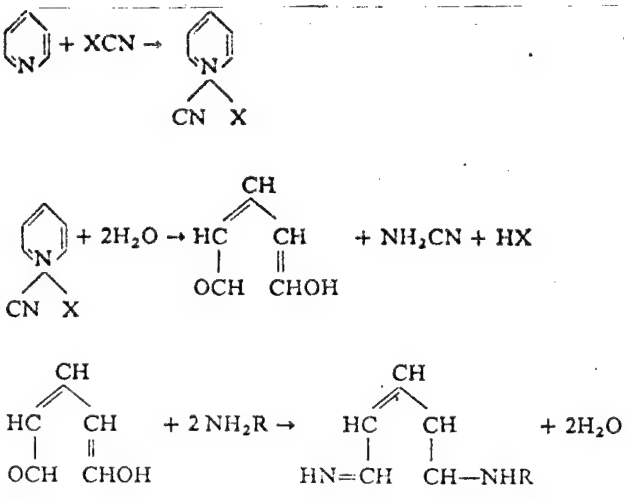


28.5.2. Qualitative Methods of Detection for Cyanogen Halides

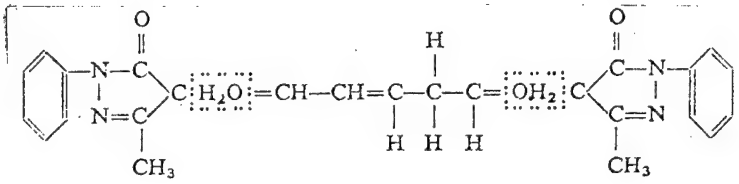
Part of the methods described for hydrocyanic acid are also suitable to detect cyanogen halides.

28.5.2.1. Reaction with Pyridine

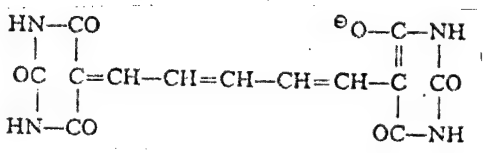
The most important methods of detection and determination for cyanogen halides and after conversion for hydrocyanic acid are based on the reaction according to Zincke/Konig [87]. Cyanogen halides react with pyridine and similar compounds with tertiary nitrogen with formation of glutaconaldehyde, which is condensed with various primary aromatic amines into polymethine dyes (Shiff Basin). For the progress of this reaction the opening of the pyridine ring is important. It takes place after adding the cyanogen halide and the change of valency of the cyclic nitrogen atom from 3 to 5 under the hydrolyzing effect of the water.



According to this principle many methods have been established, while besides different variants in the experimental conditions instead of pyridine picoline, anabasin and 4-benzylpyridine were used and as amines aniline, benzidine, o-tolidine, toluidine, p-phenylenediamine, resorcinol, dimethyldihydroresorcinol (dimedon) and barbituricacid were used. A special reagent is used by Epstein [36]. He obtained by condensation of glutakonaldehyde with 3-methyl-1-phenyl-5-pyrazolone a blue product.



The method has been modified in different ways and been rendered more sensitive allowing the determination of 0.2 µg/ml. The 3-methyl-1-phenyl-5-pyrazolone can be replaced by ethylacetonedicarbonate or ethylacetoacetate [88]. Among the many amines indicated benzidine, dimedon and barbituricacid [89] are used predominantly combined with pyridine. The use of the last reagent offers as compared with benzidine the advantage of higher sensitivity and higher stability of the color form. The sensitivity to disturbances by oxidizing substances and not for the least the cancerogenic effect of benzodine are further reasons for replacing it by barbituricacid or other suitable compounds. A reddish-violet condensation product is formed of glutacondialdehyde with 2 ml barbituricacid:



The practical accomplishment of the qualitative detection can be carried out according to the procedure described in section 28.5.3.2. for the photometric determination.

28.5.2.2. Differentiation of the Cyanogenhalides

A possibility for differentiating the cyanogenhalides is achieved by their behavior with regard to reducing agents. In acid solution cyanogenchloride is not reduced by iodide and thiosulfate. Cyanogenbromide on the other hand is reduced very quickly by thiosulfate, slowly with iodide and sulfide. Cyanogeniodide is reduced instantly by all reducing agents into cyanide.

28.5.3. Quantitative Determination of Hydrocyanicacid and Cyanide

28.5.3.1. Titration with Silver Nitrate

Cyanide can be titraded by argentometry in neutral solution according to the method of Mohr, in acid solution according to the method of Volhard or in alkaline solution according to the method of Liebig. For the last method the initial excess of cyanide causes the dissolution of silver cyanide as a complex alkali silver cyanide. The first silver excess gives a turbidity, through which the final point of titration is recognized.

The classical method is improved by the use of the Feigl silver reagent dimethylaminobenzylidenerhodanine /91/ as indicator for silver excess.

The indicators used for the argentometric determination of cyanide also includes diphenylcarbazide, dithizone, thiofluorescein and variamine blue.

$$AgCN + KCN \rightarrow K[Ag(CN)_2]$$

$$K[Ag(CN)_2] + AgNO_3 \rightarrow 2AgCN + KNO_3$$

Procedure /92/:

The phenolphthaleinalkaline cyanide solution (30 to 50 ml) is treated with 0.5 ml indicator (30 mg dimethylaminobenzylidenerhodanine in 100 ml acetone). Then the titration is carried out from a microburette with 0.01 normal silver nitrate solution until the originally yellow color assumes a reddish tinge. 1 ml of the silver nitrate solution corresponds to 0.52 mg cyanide ion. The minimum quantity which can be determined is 0.02 mg cyanide in the sediment.

28.5.3.2. - Colorimetric Determination after Conversion into Cyanogenchloride /89/

The method indicated below is one of the variants of the reagent system described in section 28.5.2.1.

Reagents:

Chloramine, 1 percent aqueous solution of monochloramine-T(B).

Pyridine-barbituricacid reagent.

In a 50 ml measurement flask 3 g barbituricacid are weighed and made into a paste with about 30 ml water, after adding 15 ml pyridine we agitate until the barbituric acid is dissolved, finally 3 ml concentrated hydrochloricacid are added and water is filled up to the mark, the reagents must be colorless.

Procedure:

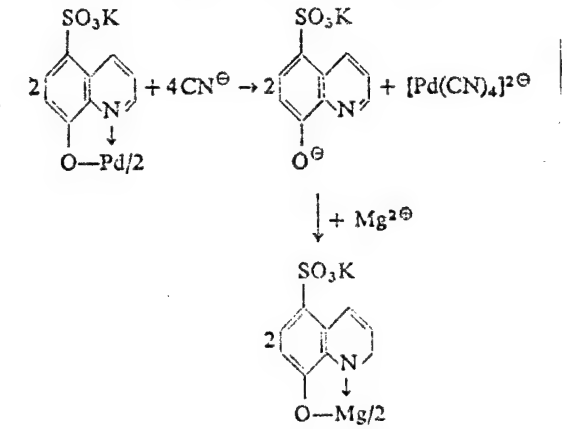
20 ml of the aqueous specimen solution (from pH 2 to 10) are treated with 1 ml chloramine solution, agitated and after 1 to 2 minutes 3 ml reagent solution are added. The reddish-violet color is photometrized after 8 minutes at 570 nm. The evaluation is carried out on the basis of the calibration curve.

With the qualitative detection in which even smaller specimen volumes can be processed in the test tube, up to 0.01 μg cyanide per milliliter can be identified. Thiocyanates and oximes represent disturbing factors in this process.

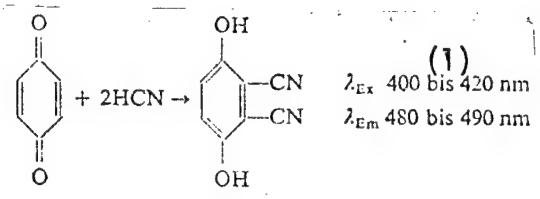
28.5.3.3. Fluorimetric Methods of Detection and Determination

The tiniest amounts (up to 0.05 μg) of hydrocyanic acid may be determined after conversion with chloramine-T into cyanogenchloride by reaction of the latter with nikotinamide in alkaline solution into a strongly fluorescent product.

The unmasking of palladiumchelate of 8-hydroxy-5-quinolinesulfonic acid by cyanide ions can be used through the formation of the strongly fluorescent chelate with magnesium to determine slow concentrations of cyanide (0.02 μg).



The formation of products with green fluorescence takes place in the reaction between cyanide ions and p-benzoquinone as well as various other quinone derivatives.



(1)

λ_{Ex} 400 bis 420 nm

λ_{Em} 480 bis 490 nm

Key: 1) to

With p-benzoquinone even 0.2 μg cyanide are detectable and with benzoquinonemonoxine-sulfonicester, 0.5 μg cyanide.

28.5.3.4. Other Colorimetric Methods

In the unmasking of the yellow palladiumchelate of 8-hydroxy-7-iodo-5-quinoline-sulfonic acid by cyanide ions in the presence of iron(III)- ions the blue color of the iron complex is produced with an absorption maximum of 650 nm.

Even 0.2 μg cyanide can be determined. Sulfide ions give a similar type of reaction.

In an indirect method mercury chloride in excess is added to the cyanide solution; the noncomplex bound mercury ions give with p-dimethylaminobenzylidenerhodanine a red color.

A colored compound suitable for colorimetric determination with an absorption maximum of 540 nm is formed in the reaction of cyanides with chloranilic acid or mercury chloranilite.

28.5.4. Quantitative Determination of Cyanogenhalides

The methods based on the formation of polymethine dyes is suitable for colorimetric determination (compare section 28.5.3.2.), while it is not necessary to add chloramines.

28.5.4.1. Determination of Cyanogenchloride by Reaction with Alkaline

The specimen solution is treated with an excess of adjusted soda lye. From the equation it is apparent that for each mol of cyanogenchloride 2 mols sodium lye must be used.

$$ClCN + 2NaOH \rightarrow NaCl + NaOCN + H_2O$$

The excess soda lye is then titrated again with sulfuric acid using phenolphthalein as indicated.

28.5.4.2. Determination of Cyanogenbromide

The method is based on the reduction of cyanogenbromide with sodiumthiosulfate in acid solution.

$$BrCN + 2S_2O_3^{2\ominus} + H^{\oplus} \rightarrow Br^{\ominus} + HCN + S_4O_6^{2\ominus}$$

Procedure:

The maximum 0.05 molar solution of cyanogenbromide in 0.5 normal sulfuric acid is quickly treated with the same volume of 0.1 normal thiosulfate solution. After adding a starch solution 0.1 normal iodine solution is added until the color becomes blue, then titrated with thiosulfate solution until discoloration.

28.6. Halogen Derivatives of Carbonic Acid

The military chemists are interested primarily in the problem of the detection of the most important representative of this group of poisons, phosgene in the air. Some detection papers, chalks and tubes are described therefore in section 32.2.

28.6.1. Qualitative Method of Detection for Phosgene

28.6.1.1. Detection with Aniline Water

In the reaction of phosgene with aniline diphenylurea is formed with a melting point of 235 degrees C.

$$COCl_2 + 4C_6H_5NH_2 \rightarrow CO(NHC_6H_5)_2 + 2C_6H_5NH_2 \cdot HCl$$

Reagents:

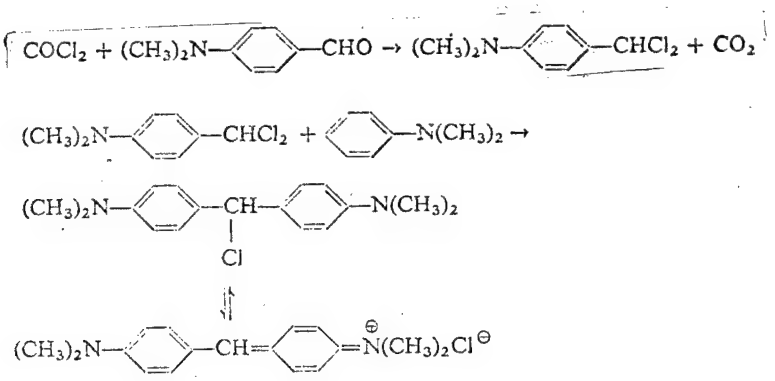
4 g aniline are agitated with 100 ml water. After 1 hour it is filtered through a filter moistened with water, the solution is treated with diphenylurea, agitated and filtered once again.

Procedure:

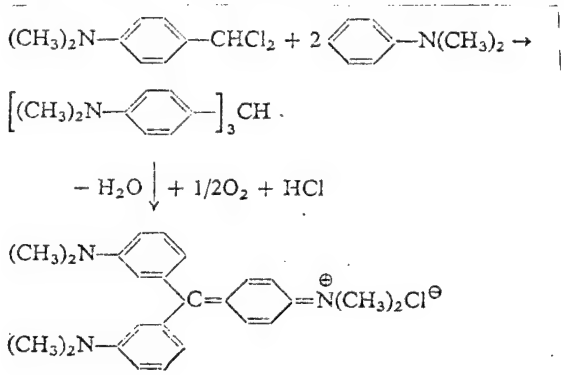
The air containing phosgene is sucked up through the reagent solution in a washing flask. A white turbidity or precipitate indicates the presence of phosgene. The limiting concentration is at 0.05 mg/ml.

28.6.1.2. Color Reaction with p-Dimethylaminobenzaldehyde and Dimethylaniline [75]

In this reaction suitable for test tubes probably a blue-green diphenylmethane dye is produced.

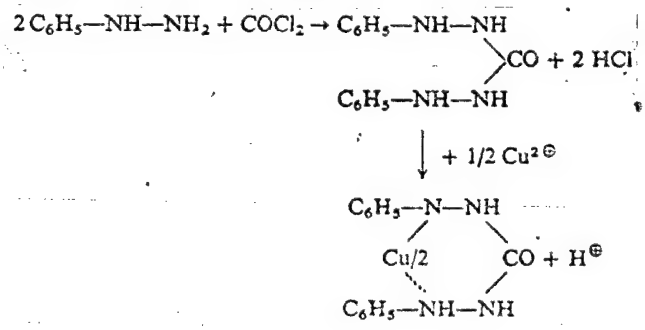


At the same time through a secondary reaction there appears to be formation of the leucobase of crystal violet, which is transformed into crystal violet by oxidation.



28.6.1.3. Detection by Formation of Diphenylcarbazide [76]

In this spot reaction from phosgene and phenylhydrazine diphenylcarbazide is formed which gives with copper salt an intensely violet internal complex.



Reagents:

Cinnamic phenylhydrazine.

Copper sulfate, 1 percent solution.

Procedure:

In the depression of a spot plate 1 drop of the phosgene solution (in ether, chloroform, carbontetrachloride) is mixed with a small grain of the cinnamic phenylhydrazine.

After 5 minutes 1 drop of copper sulfate solution is added. The presence of phosgene is indicated by a reddish-violet to pink color.

Limiting Concentration: 0.01 mg phosgene per milliliter specimen solution can be detected.

28.6.2. Quantitative Determination

28.6.2.1. Iodometric Determination

In the absorption or dissolution of phosgene in saturated acetone sodium iodide solution iodine is separated.

$$COCl_2 + NaJ \rightarrow 2\,NaCl + J_2 + CO$$

The acetone used must be dried for several days over calcium chloride, since the reaction of phosgene with potassium iodine takes place quantitatively only in the absence of water.

Procedure:

An ampule with about 0.4 g liquid phosgene is placed in a carefully dried 500 ml vessel. 5 g sodium iodide and 30 ml acetone are added to it. After sealing the flask the ampule is destroyed by fast swiveling. After violent agitation the flask is allowed to stand in the darkness for 15 minutes and then the iodine separated is titrated with 0.1 normal sodium thiosulfate solution. Parallelly the thiosulfate consumption of a control test is also determined, set up with equal amounts of iodide and acetone. The phosgene content is calculated as follows:

$$\% \text{ phosgene} = \frac{(A\text{-}B)\ 4.95 \text{ by } 100}{\text{weight in milligrams;}}$$

A = consumption of specimen of 0.1 n Na2S20 (ml)
B = consumption of the control test for 0.1 n Na2S203 (ml).

The presence of free chlorine dissolved in the phosgene falsifies the values obtained. The free chlorine is determined by the dissolution of phosgene in a soda solution, subsequent addition of sodium iodide and titration of the iodine separated after acidulation with thiosulfate.

In the determination of phosgene in the form of vapor before the absorption in the acetone free chlorine and hydrogenchloride can be removed by washing the gas current with concentrated sulfuric acid and sending it through a calciumchloride tube filled with metal antimony.

28.6.2.2. Colorimetric Determination with 4-(p-nitrobenzyl)-pyridine

Phosgene gives the reaction with 4-(p-nitrobenzyl)-pyridine [1187] a yellow to orange biquarternary ammonium salt. To carry out the determination the air containing phosgene is sent for 1 minute through 6 ml of a solution of 20 mg 4-(p-nitrobenzyl)-pyridine in methylisobutylcarbinol. After 5 minutes colorimetry is carried out at 415 nm. The calibration curve forms a straight line in the range of 0.5 to 5 µg/ml. The sensitivity of the method is 0.1 to 0.2 mg phosgene per cubic meter of air.

Phosgene can be determined with the same sensitivity in the air if the reagent used is a solution of 0.25 percent 4-(p-nitrobenzyl)-pyridine and 0.5 percent N-benzylaniline in diethylphthalate [194].

28.6.2.3. Other Methods of Determination of Phosgene

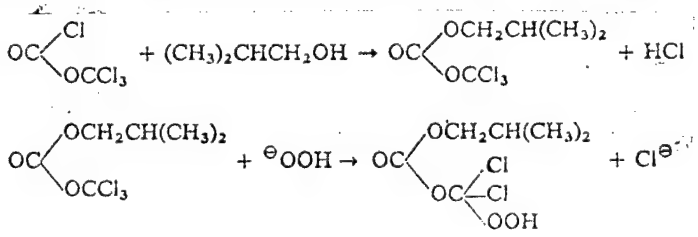
The reaction of phosgene with anilines in aqueous solution is quantitative and the deposit of diphenylurea can be weighed for the gravimetric determination. In the transfer of a current of air containing phosgene over silver wool at 800 degrees C besides silver chloride, carbon monoxide is formed which is collected in the nitrometer on potassium lye and can be measured.

In the reaction with hexamethylenetetramine bis-mexamethylene urea is formed.

After the distillation of water vapor from the alkaline solution the excess of hexamethylenetetramine is titrated with lye using a mixed indicator of methyl red/methylene blue. Chlorine and hydrochloric acid do not disturb the determination. Several older methods of determination are based on the titration of the chloride formed in the hydrolysis with soda lye with silver nitrate or the hydrochloric acid formed in the hydrolysis with water by means of lye.

28.6.2.4. Colorimetric Determination of Diphosgene [77]

In the detailed description of the Schoenemann reaction (28.1.1.1.) it was indicated that acid chlorides are also involved in this reaction. This fact may be used to determine the chlorine carbonic acid trichloromethylester (diphosgene). Reproducible results are obtained if the chemical warfare agent is dissolved in isobutanol and this solution stable for several hours is used for the subsequent determination. Trichloromethylcarbonic acid isobutylester, from which it is most probable that the alkaline hydrogen peroxide solution will give off one or several chlorine atoms with the formation of peroxide. The latter then cause the oxidation of amine



Reagents:

o-dianisidine, 1.2 percent solution in acetone, which is produced fresh everyday.

Sodiumperborate, 0.25 percent solution in water.

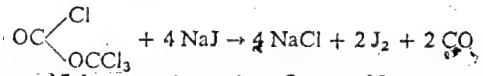
Procedure:

Three ml of the solution of the warfare agent in dehydrated isobutanol are added to a mixture of 2 ml o-dianisidine and 3 ml sodiumperborate solution. After mixing and

after 5 minutes in the colorimeter at 450 nm the extinction is measured compared with a control test. The evaluation takes place with a calibration curve, which follows in the concentration range 2 to 10 µg the Beer's law. To determine higher concentrations (40 to 120 µg) we use as oxidizable reagent 2 ml of a 0.25 percent solution of indol in acetone. After 10 minutes 15 drops of aniline are added to resolve the indigo formed; thereafter the extinction is measured at 630 nm.

28.6.2.5. Other Methods of Determination of Diphosgene and Triphosgene

Similarly to phosgene diphosgene can be determined by release of iodine from acetone-sodiumiodide solution; with triphosgene the reaction is not stoichiometric.



Both poisons react with aniline water to form diphenylurea, which can be determined gravimetrically. The conversion factor is according to the variation of the reaction 0.4661 for diphosgene and triphosgene.

The hydrolysis of both the esters with separation of 4 equivalents of hydrochloric acid for diphosgene or 6 equivalents for triphosgene takes place only slowly in cold water, but quickly in hot.

28.7. Psychotoxic Warfare Agents (Psychological Poisons).

28.7.1. Indol Derivatives

28.7.1.1. Qualitative Detection

The color reactions described for the qualitative detection of the parent grain alkaloids can also be used for the indol substances of interest in military chemistry.

Keller Reaction

This color test for parent grain alkaloids was originally carried out by dissolving the specimen substance in glacial acetic acid which contains some iron(III)-chloride and providing for this solution a sublayer of concentrated sulfuric acid. On the boundary of separation of the two phases then a blue-violet ring is produced. Rieder and Boehmer were able to prove that the glyoxylic acid contained as impurity in the glacial acetic acids plays the decisive role in the mechanism of this color reaction.

Reagent:

Glacial acetic acid containing 0.05 percent iron(III)-chloride and 0.1 percent glyoxylic acid.

Procedure [1067]:

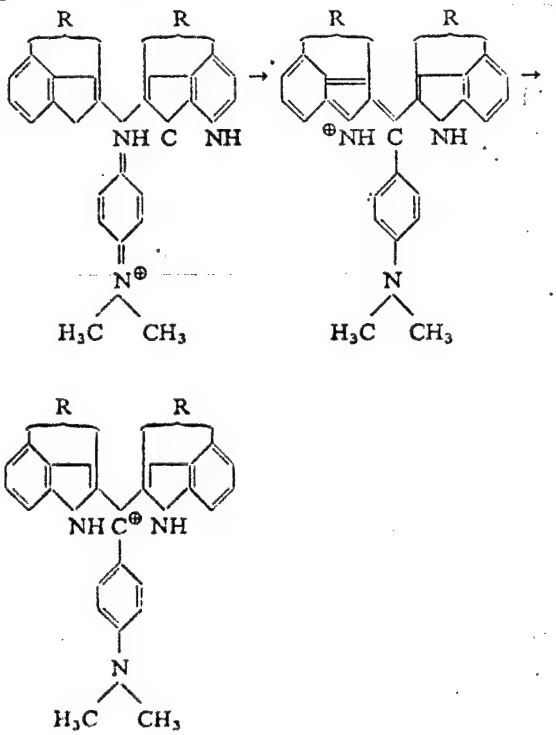
The specimen substance is dissolved in 1 ml reagent. A sublayer of concentrated sulfuric acid (free from nitrate) is provided carefully and after 15 seconds, it is agitated. LSD-25 (d-pysergic acid diethylamide) and other indol derivatives including psilocybin, dimethyltryptamine and bufotenine, give a stable blue color. This detection

reaction is characterized by the high selectivity for indol derivatives with free two position on the indol nucleus.

Color Reaction According to Van Urk/Smith [107]

All parent grain alkaloids give with p-dimethylaminobenzaldehyde a blue color in strong sulfuric acid solution. A comparison of the absorption spectra of the blue colors which were obtained on one hand with the Van Urk/Smith color reactions of the parent grain alkaloids, on the other hand with the reaction of beta-indolylacetic acid with p-dimethylaminobenzaldehyde, allows the hypothesis that the limiting forms given below are probable for the blue color salt [108].

The color reaction is specific for alpha- and beta-unsubstituted indol derivatives and has been found suitable generally for detecting the LSD.



We find the composition of the reagent solution in the description of the quantitative determination (compare section 28.7.2.2.). 0.2 ml of the alcoholic specimen solution are treated with 0.2 ml water and agitation with 1 ml of the reagent solution. A blue color appears in the presence of more than 5 µg LSD.

Detection by UV Light

All derivatives of lysurgic acid are recognizable in solution and on chromatograms by the blue fluorescence in the UV light. The UV absorption spectrum typical for these compounds and which is characterized by a smooth maximum of 316 to 318 nm and a minimum at 268 nm does not permit naturally any differentiation between the different derivatives.

28-7.1.2. Quantitative Determination

Keller Reaction

The photometric determination takes place with the modified Keller reagent modified by Rieder and Boehmer [120].

Reagents:

40 ml of a 0.25 percent solution of sodiumglyoxylate in water, 0.165 ml of a 2 percent iron(III)-chloride solution ($FeCl_3.6H_2O$) and 60 ml concentrated sulfuric acid are mixed under cooling. The reagent can be stored for about 2 weeks if kept in a dark flask.

Procedure:

3 ml of the reagent are added to 0.5 ml of the aqueous solution of the indol derivatives (1 to 3.10^{-4} M). After the mixing the reaction mixture is left for 3 minutes in a boiling water bath, then allowed to cool to room temperature and at 650 nm (LSD-25) or 540 nm (psilocybin) the extinction is measured. The evaluation takes place on the basis of a calibration curve.

Van Urk/Smith Reaction

The reagents standardized by Allport and Cocking [109] is generally applicable for the photometric determination.

Reagent:

125 mg p-dimethylamidobenzaldehyde are dissolved in 100 ml 65 percent sulfuric acid and treated with 0.1 ml 5 percent iron(III)-chloride solution. The reagent can be stored for about 2 weeks if kept in the dark.

Procedure:

0.5 ml of the alcoholic specimen solution are treated with 0.5 ml water and under cooling in the ice bath with 2 ml reagent in drops. After 1 hour standing in the dark at room temperature the extinction is measured at 550 nm against a control test. The method allows the determination of 10 to 60 µg LSD.

Fluorimetric Determination of LSD-25 [110]

The preparation of the sampling material for this most sensitive method of determination takes place as follows:

20 ml of the specimen water or an aqueous extract are extracted after adjusting the pH value to 8.5 to 9 three times, each time with 15 ml chloroform. The combined chloroform extracts are agitated for 5 minutes with 5 ml 0.01 normal hydrochloric acid. The aqueous phase is separated, alkylized with diluted soda lye and extracted with 10 to 15 ml chloroform. The solvent is evaporated in water bath and the residue dissolved in

methanol. The methanol solution is applied on a plate coated with silica gel and the substances contained in the solution are separated by thin layer chromatography. A mixture of trichloroethane and methanol (9 to 1) is used as working medium. The LSD spots visible in the UV light through blue fluorescence are eluted with methanol. For the quantitative fluorimetric measurement monochromatic ultraviolet light of wavelength 313 nm is used for the excitation, the fluorescence light is measured at 408 nm, at 445 nm in aqueous solution. In the range of 0.01 to 1 µg LSD/ml there is linear relationship between the fluorescence light and the concentration.

28.7.2. Quinuclidylbenzilate (BZ)

BZ is the benzilic ester of quinuclidinol. It is detectable analytically through known methods of detection for benzylic acid and tertiary nitrogen.

28.7.2.1. Qualitative Detection

Color Reaction with Sulfuric Acid

Von Welch and Smith [114] observed that benzylic acid produces colored cations when dissolved in concentrated sulfuric acid. This fact can be used to identify BZ in the presence of solid specimen substances on thin layer chromatograms.

Some crystals of the specimen substance are treated in a small porcelain dish with a few drops of sulfuric acid and heated. BZ causes a strong red color. It may be noted that adamsite and the alkaloid veratrine give similar colors.

Color reactions are also observed with other alkaloid reagents containing sulfuric acid. With the Marquis reagent (formaldehyde-sulfuric acid) and the Meckes reagent (selenic acid-sulfuric acid) a dark blue color is obtained, and with the Froehdes reagent (molybdate-sulfuric acid) a reddish-violet color.

Other Possibilities of Detection

a) Dragendorff reagent causes in a solution an orange-red deposit, and an orange color on the chromatogram in the presence of BZ.

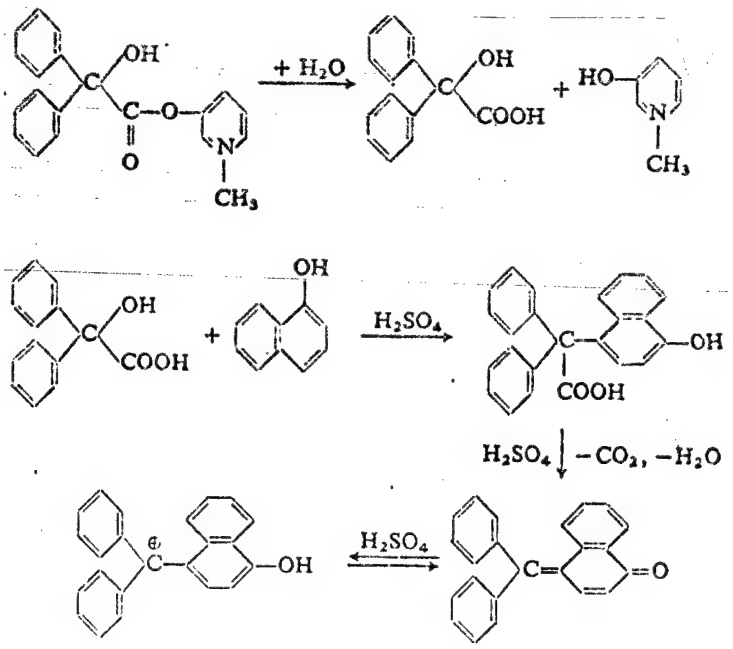
b) The naphthofuchsone reaction described in 28.7.2.2. can be used in the same way for detection. The limiting concentration is 5 µg/ml.

28.7.2.2. Quantitative Determination

Colorimetric Determination as Naphthofuchsone

The benzylic acid obtained in the alkaline hydrolysis of the BZ can be determined spectrophotometrically by a method developed by Kramer and Hackley [115]. Benzylic acid condenses with alpha-naphthol in the presence of concentrated sulfuric acid into a naphtholfuchsone dye.

The hydroxytriarylmethyl cation which has the purple color in a mixture of glacial acetic acid and concentrated acid has a high extinction coefficient at 558 nm.



Reagents:

Soda lye, 0.05 normal.

Alpha-naphthol, 0.04 g dissolved in 250 ml concentrated acetic acid.

Concentrated sulfuric acid.

Procedure:

5 ml of the alcoholic specimen solution are treated with 0.5 ml soda lye and evaporated carefully until dried. 0.5 ml naphthol solution and 1 ml sulfuric acid are added to the residue. After 5 minutes acetic acid are used to fill up to 3.5 ml and the specimen is tempered at 25 degrees C. The extinction is measured at 558 nm against a control test which was set up with the reagents. The evaluation is carried out on the basis of the calibration curve. In the qualitative evaluations there is no need of filling up with acetic acid.

28.8. Irritating Warfare Agents Which Do Not Contain Arsenic

28.8.1. Halogenated Ketones

To detect these poisons a reagent with vanilline is suitable.

Reagents:

0.1 g vanilline is dissolved in 7.5 ml concentrated acid, 2.5 ml concentrated sulfuric acid is added carefully. The reagent is freshly produced each time before use.

Procedure:

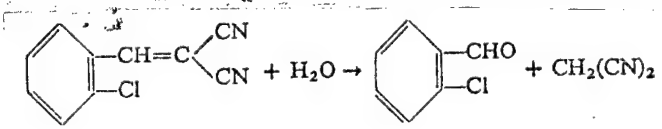
1 ml of the solution of chemical agent in carbontetrachloride is treated with 1 ml reagent and agitated. In the presence of the above-mentioned aliphatic ketones the following colors are observed:

red, gradually blue	bromomethylethylketone
red, gradually greenish-blue to greenish black	chloroacetone
green	bromacetone

When heating in water bath bromomethylethylketone and chloracetone produce a fast blue color. When the test is negative or has a slight yellowish color the three irritants are definitely absent.

28.8.2. 2-Chlorobenzylidenemalondonitrile (CS)

The white crystalline structure is easily soluble in acetone, benzol and chloroform, less soluble in alcohol, carbon tetrachloride and ether and hardly soluble in water, petroleum ether and carbondisulfide. Water hydrolyzes the compound only slowly; the addition of lyes causes a strong acceleration of hydrolysis.



28.8.2.1. Qualitative Methods of Detection

Detection with m-Dinitrobenzol

Procedure:

1 ml of the alcoholic specimen solution is treated with 0.1 ml 1 percent m-dinitrobenzol solution in alcohol and 0.2 ml 30 percent aqueous potassium lye and heated. A reddish-brown color occurring at high concentrations, and at low concentrations a color which is first reddish-violet then orange shows the presence of 2-chlorobenzylidenemalono-dinitrile. The limiting concentration is 1 µg/ml. Chloracetophenone, bromobenzyl-cyanide, and chloracetone gives similar colors (see section 28.8.3.1.).

Reaction with Quinones

The color reactions of 2-chlorobenzylidenemalonodinitrile with different quinones can be used for detection in solutions, chromatograms and in the air by means of indicator tubes. With p-benzoquinone, a blue color is found, with tetrachlorobenzoquinone (chloranil) a bluish-green color, with 1,2-naphthoquinone a reddish-violet and with 1,4-naphthoquinone a bluish-violet color is obtained.

Reagent:

Sodium 1,2-naphthoquinone-4-sulfonate, 0.002 percent in water.

Procedure:

1 ml of the specimen solution is treated with 0.5 ml reagent and 1 drop of 1 normal soda lye. A reddish-violet color which remains stable in the acidulation with acetic acid indicates the presence of 2-chlorobenzylidenemalonodinitrile. The limiting concentration is 0.001 mg/ml. The similar colors caused by higher concentrations of chloracetophenone and bromobenzylcyanide disappear in the acidulation with acetic acid.

Detection with benzofurazoneoxide solution in ethanol and 0.5 ml 1 N soda lye. In the quantitative determination subsequently water is used to fill to 10 ml. A violet color shows the presence of CS. The photometric measurement takes place after 20 to 30 minutes at 580 nm.

The reagent is suitable for detecting CS on paper and thin layer chromatograms and for application as spot test on reagent paper, while 0.5 g/cm2 can be detected.

Ultraviolet Spectrophotometric Detection

Aerosols collected on air filtering paper can be detected by ultraviolet spectral photometry at 260 nm [119].

28.8.2.2. Quantitative Determination

Colorimetric Determination with Chloranil [113]

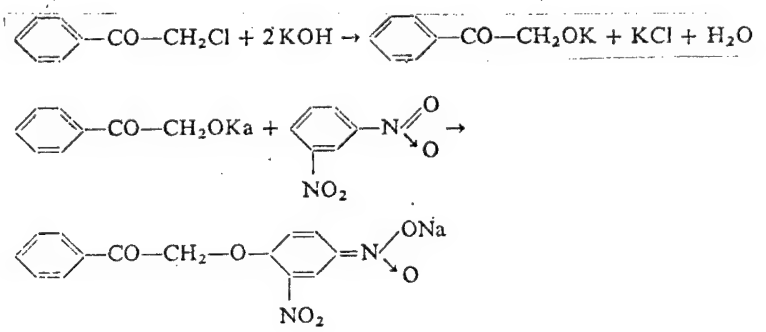
The extinction of the color which occurred in the reaction of CS or its product of hydrolysis malonic acid dinitrile with chloranil in the presence of ammonia is measured at 680 to 700 nm. The limiting concentration for CS if 2 µg/ml for malonic acid dinitrile 1 µg/ml.

28.8.3. Chloracetophenone

28.8.3.1. Qualitative Detection

Color Reaction with m-Dinitrobenzol

A general reaction for detection of compounds with active methyl group, including the chemical warfare agents chloracetophenone and bromobenzylcyanide is the color reaction with m-dinitrobenzol in the presence of alkalis [78]. The explanation for the color formation is most probably the quinoid structure in the salt of a nitrolic acid.



Reagent:

m-Dinitrobenzol, 1 percent solution in ethanol.

Potassium lye, 30 percent.

Procedure:

1 ml of the alcoholic specimen solution is treated with 0.5 ml reagent and 3 to 5 drops of alcoholic potassium lye. A reddish-violet color indicates the presence of chloracetophenone and 2-chlorobenzylidenemalonodinitrile. Bromobenzylcyanide is detected by a reddish-brown color and chloracetone by the solution becoming red. In case of negative results for the specimens, these poisons are definitely not present. A differentiation is possible by acidulation of the reaction mixture with acetic acid. The color caused by chloracetophenone disappears immediately. In the presence of the irritant CS the color is stable. Limiting concentration: 0.001 mg chloracetophenone per milliliter are detectable.

Nitration to Dinitrobenzoic Acid

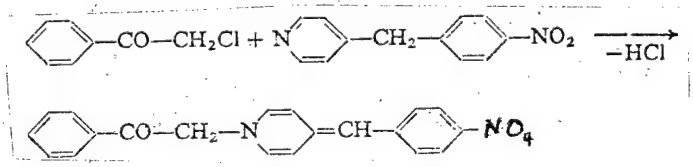
By means of nitric acid chloracetophenone can be nitrated to dinitrobenzoic acid. The latter is detectable after reduction in the form of colored ammonium salts of diaminobenzoic acid [79].

Procedure:

In the test tube a few crystals of the substance are covered with 0.5 to 1 ml concentrated sulfuric acid with the addition of a few crystals of potassium nitrate and heated with the burner for a short time until the development of brown vapors. After cooling the mixture is poured into a small beaker with 2 to 3 ml water and treated with 10 ml 15 percent ammonia and hydroxylamine hydrochloride on the tip of the spatula. Chloracetophenone is identified through a reddish-brown to red color. It may be necessary to heat briefly on the water bath..

Detection with 4-(p-Nitrobenzyl)-pyridine [80]

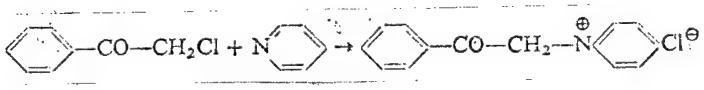
With 4-(p-nitrobenzyl)-pyridine chloracetophenone produces in ethanol solution and with the addition of dimethylformamide a red color whose production is explained by the following reaction.



28.8.3.2. Quantitative Determination

Colorimetric Determination with Pyridine [80]

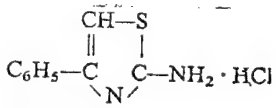
With pyridine chloracetophenone reacts with the formation of a yellow phenazylpyridium-chloride.



The chloracetophenone contained in the air as vapor or aerosol is absorbed in a pyridine-dimethylformamide mixture. By heating in the water bath the color is produced. The addition of piperidine stabilises the color which is colorimetrized after 15 minutes at 450 nm. The calibration curve shows a straight line in the concentration range of 1 to 25 µg/ml. In the absorption from 10 l air it is possible still to detect 0.6 mg/m3.

Reaction with Thiourea /807

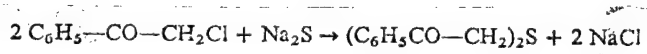
Possibilities to determine chloracetophenones are obtained through the reaction with thiourea which takes place with formation of the hydrochloride of the 2-amino-5-phenylthiazole.



The reaction which is quantitative can be evaluated by volumetric determination of hydrochloric acid or by colorimetry of the colored product occurring in the combination with diazo-p-nitraniline.

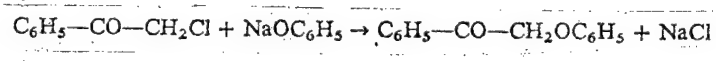
Separation of Chlorine with Sodium Sulfide.

When chloracetophenone is heated in alcoholic solution with sodium sulfide, a chloride is formed which is determined argentometrically according to the method of Volhard. Before the titration it is necessary to remove the disturbing sulfide residues by boiling after acidulation and oxidation with hydrogen peroxide.



Reaction with Sodiumphenolate

In the reaction which takes place stoichometrically with sodiumphenolate, sodium chloride is produced which is also titrated argentometrically according to the method of Volhard.



28.8.4. Bromobenzylcyanide

28.8.4.1. Qualitative Methods of Detection

Separation of Cyanide

When melting with alkalihydroxide or in the treatment with alcoholic alkaline lye in cold conditions bromobenzylcyanide gives off cyanide which can be detected by the usual methods (compare section 28.5.) The Berlin blue method is very suitable.

Reaction with o-Dinitrobenzol

In the presence of alkali with o-dinitrobenzol bromobenzylcyanide gives a colored reaction which can be used for sensitive detection.

Reagents:

o-Dinitrobenzol, 1 percent in ethanol.

Potassium hydroxide, 30 percent.

Procedure:

1 ml of the alcoholic specimen solution is treated with 0.5 ml o-dinitrobenzol solution and 1 drop of potassium hydroxide. Bromobenzylcyanide produces a violet color, which disappears in the acidulation with acetic acid.

The limiting concentration is at 0.001 mg/ml. Chloracetophenone reacts in the same way (limiting concentration 0.05 mg/ml), while o-chlorobenzylidenemalonodinitrol gives an orange color which is stable when acidulated with acetic acid.

Saponification to Ammonia

Bromobenzylcyanide is saponified under heating both with alcoholic alkali lyes and by acidulation with formation of ammonia.

$$2\,C_6H_5CHBrCN + 4\,KOH \rightarrow C_6H_5{-}C(=C_6H_5){-}COOK + 2\,KBr + 2\,NH_3$$

$$C_6H_5CHBrCN + 2\,H_2O + HCl \rightarrow C_6H_5CHBr{-}COOH + NH_4Cl$$

The ammonia can be detected with Nessler's reagent. Since ammonia is volatile in alcoholic solution, we recommend saponification with acid which is produced by concentration of the alcoholic specimen solution after adding 1 drop of concentrated hydrochloric acid, until dry. The residue is absorbed with somewhat diluted sodium hydroxide and after adding the Nessler reagent the bromobenzylcyanide is identified by the light brown color or a precipitate of the same color.

In the acidulation of the reaction mixture after the alkaline saponfication, a crystalline deposit of diphenylmaleic anhydride is formed with melting point 156 degrees C.

Other Possibilities of Detection

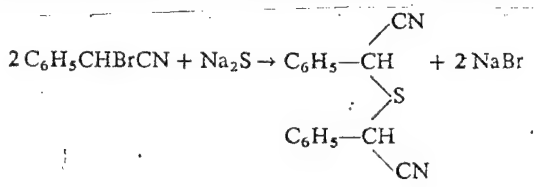
When heating a few crystals of the chemical warfare agent with concentrated sulfuric acid a brownish-red color is found of unknown origin which can be distinguished clearly from the carmine red test color obtained with adamsite. The irritant xylylbromide is indicated with a red color.

Then the chemical agent is heated with a sodium picrate solution which was obtained by dissolution of 0.5 g picric acid in 100 ml 2 normal sodium hydroxide, a red color is obtained. It is due to the formation of the sodium salt of isopurpuric acid and is obtained with all agents which give off cyanide, therefore also with diphenylcyanoarsine and tabun.

28.8.4.2. Quantitative Determination

Reaction with Sodium Sulfide

In the quantitative reaction with sodium sulfide in alcoholic solution bromine is separated as bromide with formation of bis-(phylacetonitrile)-thioether, and can be titrated argentometrically according to the method of Volhard.



The sulfide excess must be removed before the titration by boiling the solution acidulated with sulfuric acid and oxidation of the residual hydrogen sulfide with hydrogen peroxide.

28.8.5. Trichloronitromethane (Chloropicrin)

28.8.5.1. Qualitative Methods of Detection

Reduction to Nitrite

According to Aleksevskii, chlorpicrin is reduced by treatment with metal calcium or sodiumamalgam to nitrite. $CCl_3NO_2 + 8H \rightarrow HNO_2 + 3\,HCl + CH_4$

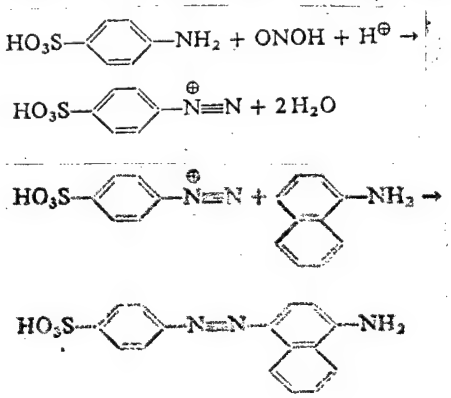
In the reaction with sodiumethylate, with methanol potassiumiodide solution or with alklye also nitrite is formed in the presence of hydrogen peroxide.

$$CCl_3NO_2 + 4\,C_2H_5ONa \rightarrow C(OC_2H_5)_4 + 3\,NaCl + NaNO_2$$

$$CCl_3NO_2 + 4\,KJ \rightarrow CJ_4 + 3\,KCl + KNO_2$$

$$CCl_3NO_2 + 6\,NaOH \xrightarrow{(H_2O_2)} Na_2CO_3 + 3\,H_2O + 3\,NaCl + NaNO_2$$

The detection of the nitrite produced is achieved with the Griess reagent, which contains alpha-naphthylamine and sulfanilic acid. The sulfanilic acid is diazotized through the nitrite, the diazonium salt is combined with the amine into a red azo-dye.



Reagents:

For reduction

Sodiumamalgam, 8 percent
metal calcium
metal sodium
powdered Devarda alloy or potassiumiodide, saturated in methanol.

Griess Reagents:

Solution A (0.1 g sulfanilic acid is dissolved in 100 ml 30 percent acetic acid)

Solution B (0.03 g alpha-naphthylamine is boiled with 70 ml water, filtered or decanted from the undissolved residue and treated with 30 ml glacial acetic acid).

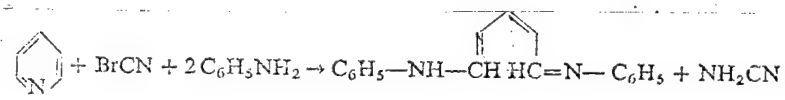
Before using equal parts of both solutions are mixed.

Procedure:

5 ml of the alcoholic solution of the chemical agents are treated with 1 ml of the reducing agent. After the reduction is completed acetic acid is used for acidulation. A red color produced after addition of 1 to 2 ml Griess reagent indicates the presence of chloropicrin.

Reaction to Thianogenbromide [81]

Chloropicrin is converted by potassiumcyanide in the presence of potassiumbromide in alcoholic solution to tetradinitropotassiumethane and cyanogenbromide. The cyanogenbromide form reacts with pyridine and aniline with the formation of a red polymethine dye. Further data on the mechanism of this reaction may be found in the description of the methods of detection for cyanichalides (see section 28.5.2.1.).



Reagents:

0.5 g each of potassiumbromide and potassiumcyanide are dissolved in 8 ml water and the solution is filled with ethanol up to 20 ml.

Pyridine

Anilinehydrochloride, 10 percent solution in water.

The complete reagent is produced each time shortly before use by mixing two parts of potassiumbromide cyanide solution and 1 part each of pyridine and anilinehydrochloride solution.

Procedure:

For the detection in the air the latter is sucked up through a washing flask filled with the reagent. Chloropicrine is according to concentration indicated by a yellow, orange, or red color. A sensitivity of 0.3 to 0.4 mg/l air is achieved.

To detect chloropicrine in water or in alcoholic extracts the following procedure is applied.

1 ml of the specimen solution is treated with 0.5 ml of KBr-KCN solution and with 0.5 ml of a mixture of 2 ml pyridine, 0.2 ml ethylacetoacetate and 8 ml water. An orange-red color indicates the presence of chloropicrin. The limiting concentration is 0.001 mg/ml.

Other Possibilities of Detection

If the air to be studied is allowed to flow through an alcoholic solution of thiophenol in the presence of chloropicrin a white turbidity or opalescence is formed, which comes from the diphenyldithioether produced. A concentration of 0.06 mg/l can be identified after suction of 2 l air after 3 to 4 minutes.

A 10 percent solution of dimethylaniline in benzol shows in the suction of air containing chloropicrin a change of color from yellow to red. By adding a few drops of hydrogen peroxide and heating the color is intensified.

With potassium salts of mercaptanes chlorpicrin reacts with formation of insoluble condensation products. This reaction can be used for detection by allowing the air to be studied to pass through an alcoholic solution of the potassium salt of dithioethyleneglycol and obtaining a yellowish deposit.

A few drops of the chemical warfare agent or alcoholic extract are boiled with 2 ml 5 percent alcoholic potassiumhydroxide and a crystal of thymol or resorcinol is added. In the presence of chloropicrin we obtain with thymol a yellow color and a red one with resorcinol. The yellow color obtained with thymol is converted into reddish-violet when sulfuric acid is added.

28.8.5.2. Quantitative Determination

Argentometric Determination After Decomposition to Chloride

The decomposition can take place by several methods. Besides the reactions with alcoholic alkali lyes and alkaline alcoholates chloride is also formed in stoichiometric quantities in the conversion with sodiumsulfite and with sodium peroxide.

$$CCl_3NO_2 + 3\ Na_2SO_3 + H_2O \rightarrow CHNO_2(SO_3Na)_2 + 3\ NaCl + NaHSO_4$$

It is advantageous to use sodium peroxide which oxidizes simultaneously all the colored organic compounds produced into colorless ones, which makes it easier to obtain the final determination of the Volhard titration.

Procedure:

In a solution of 1.5 g sodium peroxide in 50 ml 50 percent ethanol, a weighed portion of 0.1 to 0.2 g of the chemical agent is added. It is heated in the reflux cooler until it is discolored, which requires about 1.5 to 2 hours. After adding 20 ml 2 normal sodium peroxide without any cooling it is boiled for 30 minutes more. The cooled solution is acidulated with 30 ml 2 normal nitric acid, then the chloride is determined by the usual method according to Volhard.

Colorimetric Determination of the Cyanichalide

On the basis of the described method of qualitative detection two colorimetric methods were developed to determine chloropicrin in water and in the air [827].

Determination in Water

Reagent:

50 ml pyridine, 50 ml methanol and 0.6 g potassiumcyanide are agitated together with cooling under ice for 30 minutes. After filtering away the undissolved potassium-cyanide we add 0.1 g phloroglucine. The reagent must be prepared freshly each time and kept in a brown bottle.

Procedure:

5 ml of the water specimens are agitated twice each time with 3 ml ligroin. 2 ml reagent are added to the combined extract. After 5 minutes the reddish-violet color is colormetrized at 535 nm against a controlled experiment.

The limiting concentration is 0.005 mg/ml water.

Determination in the Air

Reagent:

30 ml pyridine, 70 ml methanol and 0.4 g potassiumcyanide are agitated with ice cooling for 30 minutes and treated after filtering the undissolved cyanide with 0.8 g dimedon (5,5-dimethyldihydroresorcinol).

Procedure:

The air to be studied is sucked up slowly (2 l in 15 minutes) into a washing flask filled with 5 ml methylglycol. After adding 2 ml reagent the heating is carried out in a boiling water bath for 5 minutes and after cooling the orange to red color produced is colorimetrized at 490 nm. The limiting concentration is 0.4 γg/ml solution.

28.9. Carbon Monoxide

The strong reduction capacity of carbon monoxide is the basis of most methods of detection and determination.

28.9.1. Reduction of Palladiumchloride

Carbon monoxide reduces palladiumchloride with the formation of elementary palladium.

$$Pd^{2\oplus} + CO + H_2O \rightarrow Pd + CO_2 + 2\,H^{\oplus}$$

A reagent paper impregnated with palladium chloride is colored gray to black in the presence of only 0.01 to 0.03 percent carbon monoxide in the air.

The detection becomes much more sensitive by additional use of phosphoromolybdic acid. Whereas carbon monoxide reacts only very slowly otherwise with phosphoromolybdic acid, in the presence of palladiumchloride molybdenum blue is produced immediately. The explanation resides in the adsorption and thus activation of carbon monoxide by the palladium formed. Through this heterogeneous catalysis, the reaction is accelerated.

$$2\,MoO_3 + CO \rightarrow Mo_2O_5 + CO_2$$

This system of reagents can be used in test tubes or as spot reaction for the detection of carbon monoxide which is formed in the decomposition of certain organic compounds with syrupy phosphoric acid or metal carbonyls with concentrated sulfuric acid.

Reagent:

0.02 g palladiumchloride are dissolved with 2 drops of concentrated hydrochloric acid and filled with water up to 10 ml. Of this solution 2 ml are mixed with 8 ml of a cold saturated solution of phosphoromolybdic acid in water [90, page 331].

Procedure:

A drop of the reagent solution is introduced in the decomposition vessel on a glass rod or on the stopper. After the action the drop is spread on the paper and a drop of water is applied. The blue coloring indicates the presence of carbon monoxide. Sulfur dioxide, hydrogen sulfide and hydrocyanic acid disturbs the detection. The detection with NBS indicator gel /94/ is based on the same principle. On silica gel impregnated with palladiumsulfate and ammoniummolybdate yellow silicomolybdate is formed which under the effect of carbon monoxide is reduced to the greenish-blue heteropoly compound. The sensitivity is 0.001 percent carbon monoxide. The reduction of the phosphoromolybdic acid can be used also for the photometric determination of carbon monoxide.

28.10. Fluorocarbonic Compounds

The stability of the C-F bond is characteristic of these poisons which are important because of their high oral toxicity especially for drinking water poisoning.

28.10.1. Methods of Wualitative Detection

28.10.1.1. Separation and Detection of Fluorine

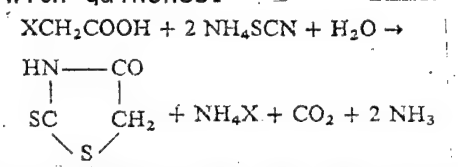
A generally applicable method to separate fluorine is heating with sodium alcoholates. To achieve a high reaction temperature and as a result shorter separation time, alcohols with 6 to 8 carbon atoms, preferably hexanol are used [103]. An exactly weighed amount (10 to 20 mg) of the fluorein compound is dissolved in a vessel provided with reflux

cooler in 15 ml hexanol and heated for 15 minutes up to boil after adding 0.2 g metal sodium. The still hot solution is extracted twice in a separating funnel each time with 10 ml water. In the aqueous extract then fluorine is determined qualitatively or quantitatively with one of the usual methods (compare chapter 26.2). To detect the vapors of fluororganic compounds in the air hexanol can be used as absorption medium in wash bottles with the subsequent application of this procedure. In the presence of fluorine compounds in the water specimens the separation of fluorine is achieved by heating with a mixture of potassiummetaperiodate, silver perchlorate and perchloric acid.

The fluorine separated is distilled away after adding glass wool.from the reaction mixture in the form of fluosilicic acid. The mineralization with calcium oxide was used to establish fluorine in fluoracetic acid, but uncontrolled losses of fluorine were observed, presumably because of the formation of fluoromethane. The digestion in the nickel cylinder according to the method of Wurzschmitt with sodium peroxide adding a little glycerine is very suitable.

28.10.1.2. Detection as Rhodanine

Feigl /93/ described a sensitive detection for monochloracetic and monobromacetic acids, which is based on a color reaction of the rhodanine formed in the heating with ammonium thiocyanate with quinones.



Under more serious conditions monofluorfluoracetic acid also reacts.

Reagents:

Ammoniumthiocyanate, 1 percent in water.
1,2-naphthoquinone, 0.05 percent in 50 percent ethanol.

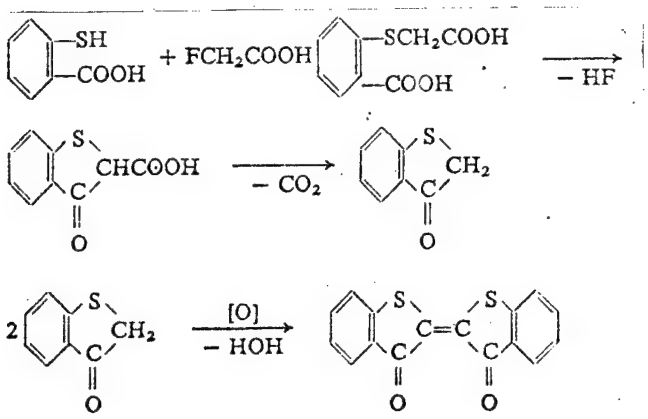
Procedure:

1 ml of the aqueous specimen solution is treated in a small beaker with 0.2 ml of the ammoniumthiocyanate solution and heated for 30 minutes in the drying case to 140 degrees C. After cooling 0.1 ml naphthoquinone solutions and 0.5 ml 0.2 percent sodium hydroxide are added to the residue. At latest after 5 minutes we compare with a control experiment set up simultaneously. A pink, red to reddish-violet color indicates the presence of fluoracetate.

The limiting concentration is 0.002 mg/ml.

28.10.1.3. Detection by Formation of Thioindigo

Similarly to the Friedlaender thioindigo synthesis in the heating of monofluoracetic acid and their salts with thiosalicylic acid and alkali, thioindoxyl is formed, which is oxidized into red thioindigo by means of potassiumhexacyanoferate (III).



A critical element in the success of the reaction is reaching a certain temperature in the melting of the alkali, for which Ramsey and Patterson /104/ considered 100 degrees C as too low and which according to experience should be about 200 degrees C. The reagents needed for achieving a qualitative detection are indicated in section 28.10.2.

Procedure:

The water specimen is adjusted by means of diluted sodium hydroxide or sulfuric acid to a pH value of 5 to 7. 1 ml of this neutralized specimen solution is treated in a small porcelain dish with 1 ml thiosalicylic acid solution and 2 drops of sodium hydroxide (1 to 1) and evaporated until dry on an asbestos wire network over a gas burner. The residue is heated further for 5 to 10 minutes and should assume here yellowish color, but should never be reddish-brown. After cooling the residue is dissolved in 1 ml potassiumferricyanide solution. In the presence of fluoracetates according to the concentration a deposit with more or less reddish-violet color is formed of thioindigo which is visible at very low concentrations often only after standing for a long time.

Detection Limit:

According to this procedure it is still possible to identify 0.02 mg sodium fluoracetate.

A higher sensitivity can be achieved if we take a larger volume of the water specimen until the alkaline reaction against lackmus with sodium hydroxide, thereafter treating with 1 ml thiosalicylic acid reagent and evaporating until dry.

28.10.1.4. Detection with Concentrated Sulfuric Acid and Chromotropic Acid

Fluoracetic acid is like all monohalogenated acetic acids hydrolyzed under heating with concentrated sulfuric acid to glycolic acid, from which under the water extracting effect of sulfuric acid, carbon monoxide and formaldehyde are formed. Formaldehyde is detected through the sensitive color reaction with chromotropic acid. A similar reaction is obtained with the chlorinated phenoxyacetic acids used as herbicides. The detection limit is 0.1 μg fluoracetic acid.

28.10.2. Quantitative Determination

After the separation or mineralization of the compounds the quantitative determination of fluoride is possible with one of the ordinary volumetric, colorimetric or enzymatic methods (compare Chapter 26).

A photometric determination /105/ of monofluoracetic acid is based on the color reaction with thiosalicylic acid (compare section 28.10.1.3.).

Reagents:

Thiosalicylic acid (1 g thiosalicylic acid is dissolved in 7 ml 4 percent sodium hydroxide; water is used to fill up to 50 ml).

Potassiumhexacyanoferrate (III), 2 percent solution in water.

Procedure:

The water specimen or aqueous extract is treated with 1 normal sodium hydroxide until alkaline reaction to lacmus and evaporated in a porcelain crucible to a volume of about 5 ml. With 1 normal sulfuric acid it is adjusted to a pH value of 5 to 7, and after adding 1 ml thiosalicylic acid solution and 2 drops of sodium hydroxide (1 to 1) we heat for 20 to 30 minutes in the water bath. Thereafter the mixture is evaporated in an oil bath at 170 to 200 degrees C until dry and heated for 30 more minutes at this temperature. After cooling the residue is dissolved in 2 ml water and treated with potassiumferricyanide solution until it becomes yellow. The content of the crucible is transferred quantitatively to a separating funnel and the thioindigo formed is extracted by double extraction with toluene. The toluene extract is filled to the corresponding volume and photometry carried out at 540 nm against a control specimen. The evaluation is carried out on the basis of a calibration curve.

28.11. Alkaloids

28.11.1. Preparation for Analysis

Alkaloids are introduced as sabotage poisons to pollute foodstuffs and fodder and drinking water, so that the specimens to be examined may occur in the following forms:

a) Foodstuff and fodder
b) Water specimens
c) Aqueous extract
d) Pure substance of alkaloids.

Alkaloids are plant bases and contain predominantly nitrogen in a heterocyclic ring system, for example pyridine, piperidine, quinoline, pyrrolidine and others. Most alkaloids are tertiary bases, but few of them are primary, secondary and quarternary bases. With few exceptions the alkaloids are solid, crystalline, colorless substances of low alkalinity.

The identification possibilities available to the analyst of chemical warfare agents include group precipitation and color reagents and after evaluating these preliminary

experiments, various special detection processes for the individual alkaloids as well as the application of chromatographic methods of separation and detection.

The preparation for analysis depends on the type of specimen material and on the desired degree of identification. With the pure substance the general color reactions are carried out and after indication of the presence of a certain alkaloid have been obtained by this means, special detection reactions for the alkaloid concerned. The procedure is the same for water specimens and aqueous extracts using group precipitation reagents. Tables 28.4 and 28.5 should be of help to evaluate the preliminary experiments.

The alkaloids must be extracted from foodstuff and fodder and isolated after separating the disturbing contents. Then the procedure according to Stas/Otto very commonly applied in toxicological analysis is used, in which the different alkalinity of the alkaloids is taken for the classification in three groups. The specimen material is crushed, suspended in alcohol and after adding some tartaric acid or a little sulfuric acid is boiled with reflux for 15 minutes. After cooling it is filtered and the filtrate is concentrated greatly in the water bath. In the absorption with cold water the fats and resins are separated. Filtering is carried out and once again evaporation. The syrupy residue is agitated with absolute ethanol, while inorganic salts, proteins, peptones and dextrines are separated. After repeated filtering, evaporation of the alkaloid and recovery of the residue with some water, the solution of the alkaloid sulfates or tartarates is obtained. Then the group separation is carried out, if necessary without further preliminary treatment the group precipitation reagents are applied. The group separation takes place by extraction from alternating acid or alkaline medium. The first group contains the alkaloid extracted from the weakly acid solution. To separate them the above solution or the water specimen treated with tartaric acid is treated first with ether then with chloroform containing 10 percent ethanol. After the evaporation of the solvent we find in the residue of the ether extract among other substances picrotoxin and cantharidin, in that of the chloroform extract, colchizin and veratrine.

The second group contains the alkaloids of strongly basic nature which can be extracted with ether only after alkalyzing the aqueous solution with sodium hydroxide. After evaporating the ether we find in the residue among other substances atropine, nicotine, strichnine, and brucine, aconitine, physostigmine, coniine, and cocaine.

The last group consists of alkaloids with free phenolhydroxyl groups which give with sodium hydroxide phenolates soluble in water and cannot be extracted with ether. But they can be extracted with chloroform if after preliminary acidulation the aqueous solution is adjusted with ammonia to a weakly alkaline reaction. When using this group separation naturally the possibly occurring chemical agents are contained in the different extracts according to their basic or acid nature and must, as in the case for example of nitrogen yperide, VX and BZ, be taken into consideration when using the precipitation reagents.

28.11.2. Methods of Detection

28.11.2.1. Determination of the Melting Point

In the presence of the pure substance the melting point is obtained as far as possible with the micromelting point equipment under the microscope. Table 28.3 indicates the

melting points of some important alkaloids.

A previous purification of the substances is advisable. It is accomplished by dissolving in alcohol, filtering and evaporation until a syrupy consistency, recovery of the residue with water, and after alkalizing with sodium hydroxide, extraction with chloroform. The chloroform is evaporated and the residue used to determine the melting point.

Table 28.3. Melting Points of Selected Alkaloids in Degrees Celsius

Aconitine	187 to 188
Atropine	115 to 116
Brucine	178
Colchizine	155
Morphine	230 (decomposition)
Picrotoxin	200
Strychnine	265 to 266

28.11.2.2. General Color Reactions

The use of the general color reactions is practical only for the pure substance of the alkaloids. For the implementation a few crystals of the specimen substance are crushed in a small porcelain dish or in the hollow of a porcelain spot plate with a few drops of reagent. The color reactions and their course are observed and the Table 28.4 shows which alkaloids show the observed reactions. The general color reagents used most frequently are given below.

1. Sulfuric acid concentrated pro analysi.
2. Nitric acid, concentrated pro analysi (D = 1.5 g.cm-3).
3. Froehdes reagent
   0.1 g sodium or 0.5 g ammoniummolybdate are dissolved under mild heating in 10 ml concentrated sulfuric acid. The reagent can be kept for about 10 days when stored in a cool place.
4. Mandelin's reagent
   0.1 g ammoniummetavanadate (free from nitric and chromic acid) is dissolved in finely ground state in 20 ml concentrated sulfuric acid. The reagent can be kept for about 10 days.
5. Erdmann reagent
   20 ml concentrated sulfuric acid are treated with 10 drops of a mixture of 10 drops concentrated nitric acid in 100 ml water.
   Certain color reactions are obtained with this reagent with all phenols. The reagent is freshly prepared each time.
6. Mecke reagent
   0.1 g selenic acid is dissolved in 20 ml concentrated sulfuric acid. The reagent can be kept for about 10 days if stored in a cool place.
7. Marquis reagent
   2 ml 30 percent formaldehyde solution are mixed with 100 ml concentrated sulfuric acid. The reagent can be kept for about a week.
   3 ml of the reagent are treated each time with a trace of the alkaloid.
8. Wasicky reagent
   2 g p-dimethylaminobenzaldehyde are dissolved in 6 ml concentrated sulfuric acid,

Table 28.4. Color Reactions of Selected Alkaloids

	Concentrated sulfuric acid	Concentrated nitric acid	Froehde reagent	Erdmann reagent	Mecke reagent cold	with heating	Wasicky reagent	Vitali reaction	Mandelin's reagent
Aconitine	yellow		yellow	bluish-yellow to yellow					brown
Atropine							reddish-violet to red	violet to red	red to yellow
Berberine	olive-green to yellow	red-brown	brownish-green	olive green to yellow brown					
Brucine		blood red to yellow	raspberry red to brownish		yellowish red	lemon yellow			red to yellow
Coniine			colorless to yellowish						
Colchizine	yellow	violet to yellow	violet to yellow	violet to yellow	lemon yellow	yellowish brown			
Curare	brown to red	violet	violet						yellow
Cantharidin					purple-red				
Digitalin (D. verum)	yellow			reddish violet					
Digitonin	red								
Digitoxin	brownish red								
Morphine		reddish yellow	violet red to green		blue to blue green	brown	bright red		red to blueviolet
Nicotine		yellowish to red					reddish brown		
Physostigmine	yellow to green	yellow	light reddish to yellow	light reddish to yellow	brownish to yellow	light brownish red	green		yellowish green
Picrotoxin	orange red								
Scopolamine			deep blue					red	
Solanine	brown, reddish violet on edge		blueviolet to greenish yellow					reddish orange	
Strychnine		yellow							orange red
Veratrine	yellow to orange green to red carmine fluorescence	light yellow	yellow to orange cherry red	yellow to orange green to red to carmine fluorescence			deep green to brown	red violet to orange	yellow to cherry red

to which 0.4 ml water are added. The reagent can be kept for about 1 day. To carry out the reaction a few crystals of the alkaloid are treated with 1 drop of the reagent on a beaker cover and carefully heated on an asbestos plate.

9. Vitali reaction
   A few crystals are treated with a few drops of fuming nitric acid and evaporated in the water bath until dry. The residue is treated after cooling with a drop of a solution of calcium hydroxide in absolute ethanol. Characteristic colors appear.
10. Ohkuma reaction [19, 20]
    5 to 15 micromoles of the specimen substance are heated with 1 ml of the 2 percent solution of malonic acid in acetic anhydride for 3 to 5 minutes at 100 degrees C. 20 µl of the reaction mixture are placed with a capillary pipette on a thin layer coil coated with silica gel. In the consideration with ultraviolet light (350 nm) we see around the application spot concentric colored rings in a pattern characteristic of the individual tertiary amines. By comparison with color photos of standard experiments it is possible to identify among others the various alkaloids and psychotoxic chemical warfare agents.

28.11.2.3. Group Precipitation Reactions

To carry them out the acidulated alkaloid solution is treated in drops with the precipitation reagent. The formation and appearance of the deposits are observed. The most commonly used precipitation reagents and the deposits obtained with them are described below:

1. Mayer reagent
   1.35 g mercury (II) chloride are dissolved in 100 ml 5 percent potassiumiodide solution. The precipitation takes place from hydrochloric or sulfuric acid solution. Amorphous or crystalline precipitates are obtained. Colchicine and solanine are not precipitated; precipitations are obtained with atropine (white, cheeselike), berberine (yellowgreen), brucine (0.02 mg/ml), coniine (amorphous), narcotine, nicotine, morphine, physostigmine, strychnine (0.07 mg/ml) and veratrine.

2. Dragendorff reagent
   8 g bismuthsupnitrate are dissolved in 20 ml nitric acid (D = 1.18) and poured into a solution of 27 g potassiumiodide in 40 ml water. It is allowed to stand for 2 to 3 days, removed from the crystallized potassium nitrate and filled up to 100 ml. The reagent is stored in a brown flask. Precipitations are obtained from sulfuric acid solution with the following alkaloids: aconitine (chromium yellow), atropine (powdery, reddish yellow, later canary yellow), berberine (orange red), colchizine, morphine (yellowish red, redissolved when allowed to stand), narcotine, nicotine (0.025 mg/ml), brucine, physostigmine, strychnine (light yellow, later darker) and veratrine (light yellow, later canary yellow).

3. Wagner reagent
   5 g iodine are dissolved in 100 ml 10 percent sodium iodide solution. Light to dark brown, mostly amorphous deposits are obtained from hydrochloric acid solution. Atropine, brucine, colchizine, coniine, heroin, narcotine, papaverine (after some time dark red needles) and strychnine give precipitations.

4. Sonnenschein reagent
   10 g phosphoromolybdic acid is dissolved in 100 ml water. The reagent gives from hydrochloric, nitric or sulfuric acid solutions amorphous yellow to yellowish brown precipitations, which assume after some time a blue or green coloring. The following alkaloids give deposits: aconitine, brucine, coniine (crystalline deposit), colchizine, narcotine, nicotine (from hydrochloric acid solution: 0.025 mg/ml), physostigmine and veratrine.

Table 28.5. Precipitation Reactions of Selected Alkaloids

	Meyer reagent	Dragendorff reagent	Wagner reagent	Sonnenschein reagent	Scheibler reagent	Gold hydrogen chloride	Platinum hydrogen chloride	Tannic acid, tannin	Picric acid	Picrolonic acid	Marmes reagent
aconitine	+	+		+		+					
apomorphine						+	+	+			
atropine	+	+	+			+	+		+		
quinine	+	+	+			+	+		+		
cocaine						+					
caffeine		+	+	+	+		+				
coniine	+		+	+	+			+		+	+
colchizine		+	+	+		+		+			
curare											
codeine	+	+									+
brucine	+	+	+	+			+	+	+		+
emetine							+		+		
hyoscyamine						+		+			
hydrastine							+		+		
berberine	+	+			+	+	+			+	+
morphine	+	+			+						+
narcotine	+	+	+	+					+		
narceine									+		
nicotine	+	+			+		+		+	+	
papaverine			+				+		+	+	+
physostigmine	+	+		+		+					+
strychnine	+	+	+		+	+	+	+	+	+	
veratrine	+	+		+		+	+	+	+		+

5. Scheibler reagent
10 g phosphorotungstic acid are dissolved in 100 ml water. Flaky, mostly yellow precipitates are obtained with the following alkaloids: berberine, coniine (crystalline), morphine, nicotine (0.01 mg/ml) and strychnine (0.005 mg/ml).

6. Marmes reagent
2 g cadmiumiodide and 4 g potassiumiodide are dissolved in 12 ml water. The reagent precipitates white, mostly amorphous deposits. Berberine (yellow), brucine, coniine (crystalline, morphine, papaverine, physostigmine and veratrine give precipitations.

7. Gold hydrogen chloride
6 to 10 g gold chloride are dissolved in 100 ml diluted hydrochloric acid. White to gold-yellow, mostly crystalline deposits are obtained with the following alkaloids: atropine (recrystallized from hydrochloric acid melting point 138 degrees C), aconitine (amorphous), berberine (orange red), colchizine, cocaine (light yellow), physostigmine, papaverine (melting point 196 degrees C), strychnine (orange) and veratrine (needles from A. melting point 182 degrees C decomposition).

8. Platinum hydrogen chloride
10 g platinum chloride are dissolved in 100 mg dilute hydrochloric acid. From the hydrochloric acid solution with almost all alkaloids bright yellow to orange, crystalline deposits are obtained, whose melting points are characteristic.

9. Picric acid
A cold saturated solution of picric acid is used in water. Amorphous narcotine and veratrine, crystalline brucine (melting point 220 degrees C), papaverine, strychnine and nicotine (melting point 218 degrees C) are precipitated as picrates. Atropine picrate (melting point 175 to 176 degrees C) precipitates from the concentrated solution. The precipitations take place from sulfuric acid solution.

10. Picrolonic Acid
2.64 g picrolonic acid are dissolved in 100 ml ethanol. With most alkaloids yellow to red crystalline deposits are obtained.

11. Tannin
A freshlyprepared 5 percent solution of 5 g tannin (tannic acid) in water is used. Amorphous, white to yellowish deposits are obtained.

12. Godeffroy's reagent
5 g silicotungstic acid are dissolved in 100 ml water. Some alkaloids, for example atropine (0.07 mg/ml) are precipitated with high sensitivity.

28.11.2.4. Special Detection Methods for Some Alkaloids

These detection methods are used after carrying out the general precipitation and color reactions indications of the presence of a certain alkaloid are obtained.

Detection of Aconitine

A few milligrams of the specimen substance are treated in a small porcelain dish with 4 drops 80 percent sulfuric acid and heated for 5 minutes in the water bath. After

adding a few crystals of resorcinol heating is carried out again. A yellowish-brown color reaching the maximum after 20 minutes indicates the presence of aconitine. The detection limit is 0.1 to 0.5 mg.

Detection of Atropine

The color which is first violet and later red obtained in the reaction according to Vitali is characteristic of atropine, since other alkaloids all give immediately a red color.

Detection of Colchizine

About 10 mg of the specimen substance are dissolved in 2 ml 1 to 1 diluted hydrochloric acid and treated with 2 drops of 5 percent iron(III)-chloride solution. When heated in the water bath colchizine gives an olive-green color which becomes even darker after cooling. If the solution is extracted with chloroform it becomes garnet red, and orange with smaller amounts of colchizine. The detection limit is 2 to 5 mg.

Detection of Nicotine

3 ml of the aqueous specimen solution are treated with 0.1 ml 1 percent potassium cyanide solution and 0.5 ml 1 percent monochloramine solution. After 1 minute 1 ml 1 percent barbituric acid solution is added. Nicotine bases give a red to reddish-orange color of the solution. The detection limit is 0.005 mg.

Detection of Picrotoxin

1 to 2 drops of concentrated nitric acid are added to a few milligrams of the specimen substance and it is evaporated until dry on the water bath. The residue is recovered with 2 to 3 drops sulfuric acid. With careful addition of a few drops of 30 percent sodium hydroxide a brick red color indicates the presence of picrotoxin.

Detection of Strychnine

Some crystals of the specimen substance are dissolved in a small porcelain dish in 1 ml concentrated sulfuric acid. A small crystal of potassium dichromate is added and the dish is moved. If strychnine is present we observe when sulfuric acid flows away from the potassium dichromate violet to blue-violet strips starting from the latter. The reaction occurs for 0.001 mg strychnine, it is prevented with nitric acid or nitrates. Aconitine gives a blue to bluish-green color.

28.12. Phytotoxic Substances

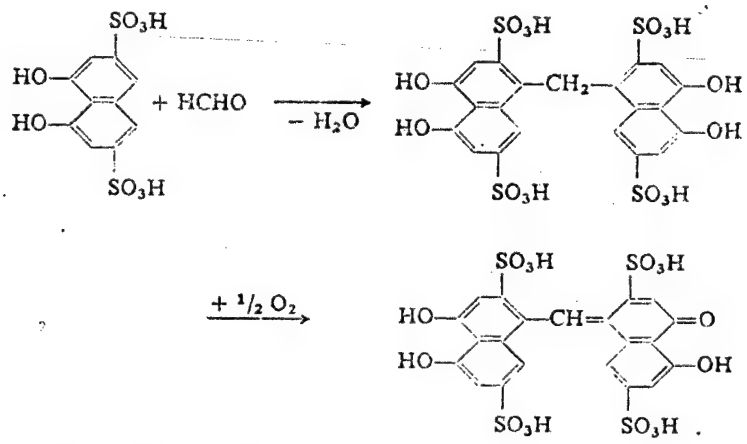
Among the many known phytotoxic compounds the substances of interest in the sector of analysis are the poisons of military significance especially those used by the American Army in South Vietnam for defoliation in jungle warfare and to destroy cultivated plants such as the chlorinated phenoxyacetic acids, the dimethylarsenic acid (cacodylic acid) N-(4-chlorphenyl)-N,N-dimethylurea (monuron), 5-bromo-3-sec-butyl-6-methyluracile (bromocil) and the 4-amino-3,5,6-trichlorpicolinic acid (picloram, tordon).

The use of phytotoxic substances was accomplished mainly with mixtures (agents "white", "orange" and "purple"); to study them it is advantageous to use chromatographic methods.

The detection and determination with chemical methods are naturally carried out more simply in the presence of pure agents. But plant, soil, and water specimens are mainly sent for examination with a very low content of plant poison. A thorough description of the problems arising in the preparation of these sample materials for analysis would go beyond the scope of this book. Insofar as the indications provided below as well as in section 32.7 (Preparation of the Specimens) are not sufficient, one must refer to specialized literature [310,311].

28.12.1. Chlorinated Phenoxyacetic Acids

2,4-dichlorophenoxyacetic acids (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and other herbicides of this group of compounds give off formaldehyde under the effect of concentrated sulfuric acid with heating. Sensitive methods of detection and determination for these phytotoxic compounds are based on their color reactions with different naphtholsulfonic acids. The course of the reaction of formaldehyde with chromotropic acid (1,8-dihydroxynaphthaline-3,6-disulfonic acid) in highly sulfuric acid solution may be explained by the following mechanisms.



28.12.1.1. Qualitative Detection

A few milliliters of the specimen solution or the extract are evaporated carefully until dry in a test tube. A few grains of I-acid (6-amino-1-naphthol-3-sulfonic acid) and 5 ml concentrated sulfuric acid are added to the residue. By careful rotation in the frame it is heated for 5 minutes up to about 170 degrees C. After the cooling a mixture of 2.4 ml water and 0.6 ml acetic acid are added. A blue color indicates the presence of chlorinated phenoxyacetic acids. The detection limit is 0.001 mg. A similar color reaction is obtained with fluoracetic acid. In the presence of esters or ester mixtures of the chlorinated phenoxyacetic acids the latter must be saponified by adding sodium hydroxide in the evaporation.

An enrichment of low concentrations from water samples is possible by extraction of the water acidulated with 1 normal hydrochloric acid with a mixture of ethyl ether and chloroform (1 to 3). The limiting concentration can be reduced through this extraction to less than 5 by 10-5 mg/ml. A quantitative enrichment from water specimens can be

achieved by using basic ion exchanges such as Dowex Amberlite XAD-2 (divinylstyrol-polymer) /304/ or Wofatit SBK. The adsorption takes place from acid solution at pH 2.0; the elution is carried out with acetone or 0.5 M methanol sodium hydroxide. Using a 200 ml water specimen, an exchanger column of dimensions 10 by 30 mm and their elution with 4 by 10 ml solvent (5 ml/minute) the limiting concentration is 2.5 by 10-2 mg, 2,4-D or 2,4,5-T per liter water.

28.12.1.2. Quantitative Determination [309]

The formation of a colored quinoid compound for the colorimetric determination of chlorinated phenoxyacetic acids is caused by the reaction of formaldehyde separated by means of concentrated sulfuric acid with I-acids.

Procedure:

A certain volume of the solvent extract is evaporated until dry in a small vessel on the water bath. The residue is treated with 3 ml of a 0.3 percent solution of I-acid in concentrated sulfuric acid and heated for exactly 5 minutes in the oil bath at 165 degrees C. The cooling to room temperature is carried out quickly and it is transferred into a 25 ml standard flask, containing 15 ml 20 percent acetic acid. It is washed with more acetic acid and filled up. The indigo blue color is photometrized at 580 nm. 10 to 100 µg, 2,4-D or 2,4,5-T can be determined.

Ether and benzol extracts from plants or foodstuffs are purified with aluminum oxide or florisil columns to separate the extracted impurities which were carbonized with concentrated sulfuric acid.

28.12.2. Cacodylic Acid

The detection of the cacodylic acid contained in the "agent blue" in a mixture with a sodium salt is carried out similarly to the general detection of chemical warfare agents containing arsenic (section 28.4.) after oxidative treatment with a common arsenic detection process.

28.12.3. Substituted Urea Herbicides

The detection and photometric determination of the substituted urea herbicides takes place generally by means of acid hydrolisis, diazotation of the aniline derivatives obtained and combination with a dye. Katz [305] gave the following procedure for the determination in water:

100 ml water are extracted with 5 times 50 ml chloroform. The solvent is evaporated and the residue heated for 1 hour with reflux with 15 ml 6 normal hydrochloric acid. After cooling it is diluted with 75 ml water and treated with 1 ml 2 percent sodium nitrite solution. The excess of nitrite is destroyed after 10 minutes by adding 1 ml 10 percent ammonium sulfamate solution. After adding 2 ml of a 1 percent solution of N-(1-naphthyl)ethylenediamine dihydrochloride it is allowed to stand for 15 minutes and the dye obtained is extracted after adding 25 g sodium chloride with 10 ml N-butanol. The organic phase is separated by centrifuging and photometry carried out at 555 nm. Calibration curves were plotted with the pure agents or with the corresponding aniline derivatives, for example chloraniline in monuron. The detection limit is 0.05 mg/l.

28.12.4. Picloram

The herbicide picloram is extracted from soil specimens with 1 normal ammoniumacetate solution. The colorimetric determination /308/ must be carried out with the exclusion of all ultraviolet radiation with a yellow light. After adding 5 times the amount of 7 normal sulfuric acid and the same amount as 0.1 m sodium nitrite solution it is mixed carefully and after 10 minutes the extinction measured at 405 nm.

28.13. Inorganic Poisons

The testing for toxic cations and anions is necessary when examining poison drinking water as well as poison foodstuff and fodder. Some of these inorganic poisons are formed in the destruction processes occurring in the sample material from warfare agents, for example arsenite and arsenate from agents containing arsenic, lead from lead tetraethyl or iron and nickel from carbonyls. The detection of inorganic poisons takes place in the aqueous extract. For field laboratory workers the drop detection processes are particularly desirable with the most specific possible reagents which would allow the omission of the prior separation of cations or anions.

In suitably erected laboratories it is possible to remove the interferences which are partly difficult to exclude by using different methods of isolation, such as the separation process, chromatography and the annular furnace technology. But it is quite possible that one of the two last methods suitably modified should be available also for field analysis in the foreseeable future. Such a prospect is offered for example by the rapid development of the thin layer chromatography method and their aids (compare section 31.3). The further development of the detection processes suitable for field and laboratory may be found in particular in the facilitation of the procedure by the use of stable reagent paper impregnated with reagents. To locate the reaction products at the site of production and increase the visibility reagents which are difficult to dissolve or insoluble in water are used preferably. Insofar as such suitably sensitive reagent papers are available, the advantage of spot methods with regard to saving of time, convenience and the low instrument requirements are obvious.

28.13.1. Toxic Cations

28.13.1.1. General Detection

Part of the cations concerned can be precipitated as sulfides. The unpleasant work with hydrogen sulfide gas is eliminated by using "solid hydrogen sulfide" which means compound which in acid solution give off hydrogen sulfide. Suitable substances are thioacetamide, ammoniumthiocarbaminate, ethyldithiocarbaminate, thiourea, thioformanilide, and various mercaptans.

Procedure:

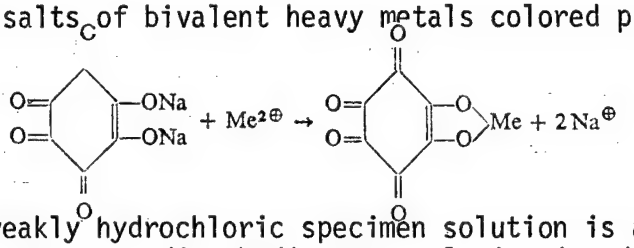
The specimen solution should be 0.25 to 0.5 normal in hydrochloric acid. Thioacetamide is added with the tip of a spatula (20 to 30 mg) to 5 ml and heated for 10 minutes in a boiling water bath. If there is no deposit the substance is treated with an ammonia solution until a pH value of 0.5 and heated once again for 10 minutes.

The following sulfides are deposited: HgS, PbS, PbS2 (black); CdS, As2S3, As2S5 (yellow) and Sb2S3, Sb2S5 (orange).

28.13.1.2. Detection of Barium

Reaction with Sodiumrhodizonate [95]

This reagent gives with salts of bivalent heavy metals colored precipitates.



One drop of neutral or weakly hydrochloric specimen solution is applied on filter paper and a drop of 0.2 percent sodiumrhodizonate solution is added. A reddish-brown color indicates the presence of barium. The limiting concentration is 2 mg/l. Lead, silver, cadmium, thalium, and strontium also form with rhodizonate colored compounds so that the detection is practical only in the absence of these cations.

Precipitation as Sulfate [96]

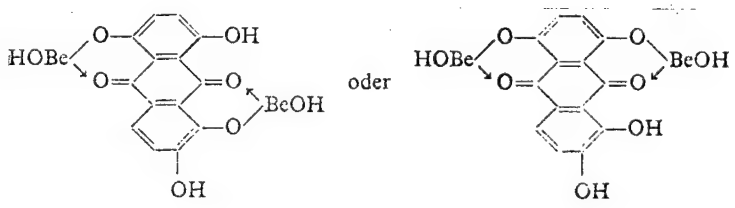
Barium sulfate has besides lead sulfate the characteristic property to include in precipitation the potassium permanganate present in such a way that the latter is no longer attacked by reducing agents.

1 drop of specimen solution is treated with 3 drops of cold saturated potassium permanganate solution and a few drops of dilute sulfuric acid. When hydrogen peroxide, oxalic acid or sulfurous acid are added the color disappears. A violet precipitate depositing indicates the presence of barium. The limiting concentration is 100 mg/l.

28.13.1.3. Detection of Beryllium

Reaction with Quinalizarin [98]

Beryllium salts form in weakly alkaline solution with quinalizarin (1,2,5,8-tetrahydroxyanthraquinone) a bluish-violet colored lacquer to which the following structure is attributed:



Reagent: quinalizarin, 0.05 percent in 2 normal ammonia solution.

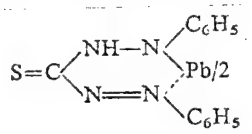
Procedure:

A drop of the specimen solution is treated on a spot plate with a drop of freshly prepared reagent solution. A blue color and in larger quantities a blue deposit

indicates the presence of beryllium. The limiting concentration is 1 mg/l. A similar colored lacquer formed in the presence of magnesium is totally discolored with the addition of saturated bromine water. Iron(III)salts must be masked by adding tartarate.

28.13.1.4. Detection of Lead

With dithizone lead salts give a red internal complex salt:



Since many other cations give complex salts of similar color with dithiazone they must be masked by adding cyanide and tartarate.

Reagent:

Dithiazone, 1 to 2 mg are dissolved in 100 ml carbontetrachloride.

Procedure:

1 drop of the neutral specimen solution is treated in a microtest tube with 1 drop each of 0.05 percent potassiumcyanide and seignette salt solution and then 1 drop of dithiazone solution and agitated thoroughly. The green reagent solution assumes a brick red color in the presence of lead. The limiting concentration is 1 to 2 mg/l.

From neutral or acetic acid solution lead is precipitated with the addition of alkali dichromate as yellow PbCrO4.

28.13.1.5. Detection of Thallium

The disturbances by lead and mercury in the precipitation of thallium as iodide are prevented by the addition of thiosulfate, which allows lead iodide to be dissolved as a complex anion, and using an excess of potassium iodide, which causes the dissolution of mercury into (II)iodide as K2(HgI4).

Procedure:

1 drop of the weakly acid specimen solution is mixed with 1 drop of a 10 percent potassium iodide solution and 2 drops of a 2 percent sodium thiosulfate solution on a beaker cover. A yellow deposit which can be recognized well against a dark background indicates thallium. The limiting concentration is 10 mg/l. If thallium(III) salts are present after iodide precipitation an iodine separation is carried out.

28.13.1.6. Detection of Mercury

Dithizone gives with mercury(II) salts a yellow complex salt.

Reagent:

Dithizone, 0.01 percent solution in chloroform.

Procedure:

1 drop of 10 percent chelaplex III solution, 1 drop of 0.5 normal hydrochloric acid and 2 drops of reagent are added to 1 drop of the specimen solution in a microtest tube. The agitate, while in the presence of mercury the chloroform layer assumes a yellow orange color. The concentration limit is 10 mg/l.

A very simple method of detection can be achieved with a piece of aluminum foil which was blank corroded beforehand with sodium hydroxide. A drop of specimen solution is applied on the washed and dried foil and after 5 minutes removed again with a piece of filter paper. The presence of mercury is indicated by a white "wax" forming after a few minutes on the foil. The concentration limit is 2 mg/l.

28.13.1.7. Detection of Cadmium

The precipitation as yellow cadmium sulfide can be considered as a specific method of detection if the cation which also formed colored sulfides can be masked with cyanide.

Procedure:

1 drop of specimen solution in a microtest tube is treated with 1 drop of concentrated ammonia solution and 1 drop of 20 percent potassium cyanide solution. The solution must be clear and colorless, if not more cyanide is added. 1 drop of a solution is applied on a filter paper and treated with 10 percent sodium sulfide solution. A yellow spot or ring indicates the presence of cadmium. The limiting concentration is 100 mg/l.

28.13.1.8. Detection of Antimony

Antimony (III) salts reduce in acid solution phosphomolybdic acid to molybdium blue /100/. The detection is specific in the absence of tin(II) salts. If work is being carried out with the hydrochloric acid solution of the sulfite precipitate, tin occurs as tetrachloride and does not disturb the detection.

Reagent paper:

Filter paper is soaked with a 5 percent aqueous solution of phosphoromolybdic acid and dried.

Procedure:

1 drop of the hydrochloric acid specimen solution is applied to the reagent paper and the latter held over water vapor. A blue color arising after a few minutes indicates the presence of antimony. The limiting concentration is 1 mg/l.

28.13.2. Toxic Anions

28.13.2.1. Detection of Fluoride

Detection processes for fluoride are described in section 26.1. The glass etching test is very simple to implement [101].

Procedure:

A few grains of potassium dichromate are dissolved in 1 to 1.5 ml concentrated sulfuric acid in a test tube. It is rotated until the wall of the glass is totally degreased and the mixture uniformly wet. A few grains of the solid or 1 drop of the liquid specimen are added and it is heated. With repeated rotation of the glass a nonuniform movement of the sulfuric acid and the formation of nonmoistened areas indicate the presence of fluoride. 0.5 µg fluorine can be detected.

28.13.2.2. Detection of Nitrite

The detection of nitrite takes place with the Griess reagent described in the detection of chloropicrin and which detects still 0.01 µg nitrite.

28.13.2.3. Detection of Arsenite and Arsenate.

In general arsenites and arsenates are detected with the Gutzeit test described in the detection of the chemical warfare agents particularly arsenic. In acid solution arsenites and arsenates are reduced, whereas in the heating of the sodium hydroxide solution with metal aluminum only arsenites are reduced to hydrogen arsenide. Arsenates and antimony compounds which form in acid solution with nascent hydrogen SbH_3 remain unchanged. If the arsenic compounds are detected besides antimony, the arsenate must be transformed in acid solution into arsenite with sodium bisulfite; thereafter reduction is carried out in alkaline solution. In the absence of other substances consuming iodine, in a solution containing bicarbonate or acetic acid, the discoloration of the iodine solution or blue iodine-star solution is characteristic of arsenites..

Arsenate gives when treated in acetic acid solution with 1 percent silver nitrate solution, a reddish brown deposit of silver arsenate.

$$AsO_4^{3\ominus} + 3\,Ag^{\oplus} \rightarrow Ag_3AsO_4$$

28.13.2.4. Detection of Selenite

By heating or evaporation with concentrated hydrochloric acid selenic acid and selenates are reduced quantitatively to selenites. Selenites accelerate catalytically the reduction of methylene blue by alkalized sulfides; this effect is used for a sensitive detection [102].

Procedure:

1 drop of specimen solution is placed in the depression of a spot plate; 1 drop of water in the neighboring depression. Thereafter in each cavity 1 drop of 0.2 molar sodium sulfite solution and 1 drop of 0.01 percent methylene blue solution are added. A faster discoloration as compared with the control specimen indicates the presence of selenite in the specimen. The limiting concentration is 1 mg/l.

Check Questions

1. Establish the reaction scheme for the first part of the Schoenemann reaction, that is the conversion of phosphoroganyls with hydrogen peroxide in alkaline solution.

2. Explain the effect of the sequence and type and method of adding the reagents on the sensitivity of the Schoenemann reaction.

3. What fluorescent reaction products are formed in the oxidation of indole by perphosphonic acid?

4. Give other analytically important reactions of organophosphoric chemical warfare agents.

5. What are the possibilities for identification of the organophosphoric warfare agents of the type VX using the chemical and biochemical methods?

6. What is the basis of the principle of the hydrolytic method for the volumetric determination of the fluorophosphororganic warfare agents?

7. What is the course of the reaction between sulfur yperite and thymolphthalein in alkaline solution?

8. Which derivatives of sulfur yperite are suitable for its identification?

9. How is the determination of sesquiyperite possible in mixtures with sulfur yperite?

10. Name some reagents which give with nitrogen yperite a characteristic precipitation?

11. What chemical warfare agents give with 4-(p-nitrobenzyl)-pyridine a colored reaction product? Establish here the general reaction equation.

12. Establish the equation of the reaction for the conversion of hydrogen arsenide with mercury bromide.

13. What products are formed in the reaction of warfare agents containing arsenic with hydrogen sulfide?

14. Why must alpha-lewisite be treated with strong alkaline lye before the detection with the Ilosvay reagent?

15. Name the specific detection process for adamsite.

16. What is the course of the Berlin blue reaction specific for cyanide ions?

17. What is the basis of the formation of the blue color in the reaction of cyanides with copper (II) salts and benzidine?

18. How is the benzoin condensation promoted catalytically with cyanide ions used analytically?

19. Establish the general reaction equation for the course of the reaction of cyanic halides with pyridine and primary aromatic amines.

20. What possibilities of detection are there for BZ?

21. How can CS be detected analytically?

22. Give a reagent generally suitable for the detection of warfare agents with active methyl group and describe the mechanisms of the reaction with chloracetophenone.

23. On what known dyestuff synthesis is the detection of fluoracetate with thiosalicylic acid based?

24. How must the group separation of alkaloids be carried out?

25. Name some color and group precipitation reactions to alkaloids?

26. What compounds are called "solid hydrogen sulfide"?

29. Biochemical Methods

29.1. Advantages of the Use of Biochemical Methods

Enzymes are used increasingly as analytical aids. This development was rendered possible through the enormous advances in the investigation of the mechanism of action and the structure of these biological catalysts. Basically enzymes act in the same way as other catalysts, but for them the catalytical processes are organized in a much higher manner. This may be seen in the high speed constance and low activation energies. Whereas many inorganic catalysts have sufficient activity only at high temperatures, the enzymes show a high activity already at physiological temperatures.

Today enzymes are available to the analyst in a constantly increasing number and with constantly increasing purity for implementing in vitro reactions for analytical evaluation. The advantages of the analytical application of enzymes are the high functional selectivity and sensitivity.

Because of the complicated spatial structure of the enzymes only certain substrates or substrate groups can reach the center of action. Emil Fischer had already established that the enzyme and substrate must fit each other like lock and key. This model representation must naturally be changed a little today since the enzyme is not a rigid matrix, but can change its spatial structure under the effect of the substrate. Through this property of the enzymes it is possible to detect specifically individual substances from mixtures of substances, which allow the omission of long separation processes and reduces the time needed for the analysis.

The catalytic effect of the enzymes and the high number of changes in the separation of the substrate gives the biochemical methods of analysis a sensitivity which lies far above the average analytical scale. By a number of changes we mean the number of substrate molecules which are separated in 1 minute from an enzyme molecule. Thus for example 1 molecule of acetylcholine esterase gives off in 1 minute 1.5 by 10exp7 molecules of acetylcholine. Since on the other hand 1 molecule of an inhibitor can always inhibit completely an enzyme molecule it is possible to derive the extraordinarily high sensitivity of the enzymatic method to determine the organophosphoric inhibitors.

The biochemical methods of analysis have three areas of application:

a) Determination of the activity of the enzymes;

b) Determination of the substrates;

c) Determination of the enzyme inhibitors (inhibiting substances).

Within the framework of the analysis of warfare agents methods for determining the activity of certain enzymes for purposes of diagnosis of poisoning and methods to determine the enzyme inhibiting warfare agents are of particular interest.

The investigations of the physiological effect of warfare agents disclose the fact that in most cases the inhibition of enzyme systems is the cause of the specific physiologic activity. Part of these in vivo effects [2] can also be established in the in vitro

[2] in vivo = literally "in life" which means in the living organism.

Table 29.1. Inhibiting Effect of Chemical Warfare Agents on Enzymes[3]

Enzyme (Source)	Inhibitor	pI50	Substrate
AChE(different)	organophosphoric warfare agents	a few up to 10	acetylcholine-chloride
SChE(different)	organophosphoric warfare agents	a few up to more than 9	butyrylcholine-iodide and others
Aliesterase (horse liver)	sarin	7	3,5-dichlorindo-phenylacetate
ATPase	soman	4	
β- and γ-chymotrypsin	DFP	about 5.3	
Lipase (milk)	DFP	5.3	
Lipase (pancreas)	paraoxon	5.2	
Aliesterase (liver)	DFP	about 6.5	
Esterase (serum)	paraoxon	about 8	
Dehydrogenase (brain)	DFP	about 4	
Phosphatase (kidneys)	DFP	about 3	
Thrombin	DFP	4.5	
Succinatedehydrogenase	soman	3	
Catalase	organophosphoric insecticide		hydrogen peroxide
Urease			urea
Carboxylase			beranzoracnic acid
Pyruvatoxidase (brain)	lewisite		
Succinoxidase	lewisite		
Pyruvatoxidase	sulfur yperite		
Hexokinase (yeast)	sulfur yperite		
Creatinphosphokinase	sulfur yperite		
Pepsin (pig)	sulfur yperite		
Pyrophosphatase (kidney)	sulfur yperite		
Cholinoxidase	sulfur yperite		
Serumpeptidase	sulfur yperite		
Succinyldehydrogenase	sulfur yperite		
Lipase	sulfur yperite		
Xanthinoxidase	sulfur yperite		
Hexokinase	irritants		
Urease			
Pyruvateoxidase			

[3]Established according to the literature data.

Table 29.2. Differentiation of Cholineesterases

	Acetylcholin-Acetylhydrolases	Azylcholin-Azylhydrolases
Synonyms	Acetylcholineesterase (AChE), specific cholineesterase, real cholineesterase, erythrocyte-cholineesterase (EChE), cholineesterase I	Cholineesterase (ChE), non-specific cholineesterase, pseudocholineesterase, serum cholineesterase (SChE), cholineesterase II
occurring mainly	blood (erythrocytes), brain, nerve ends, electric organ of the electric eel (electrophorus electricus), snake venom	blood (serum)
molecular weight	approximately 260,000	different data from 200,000 to 350,000
activity per mole	1.5 by 10exp7	5 by 10exp4 SChE (man)
points of action (number of changes)	6.1 by 10exp5 (electric eel)	
optimum pH[4]	8.0 to 8.5	8.2 5o 8.5
relative activity in the series of homolog o-azyl-cholines	acetyl propionyl butyryl choline	butyryl propionyl acetyl choline
inhibition by substrate excess	inhibition by 3 by 10-3 M acetylcholine	still no inhibition with 10-1 M acetylcholine
causes hydrolysis of:		
tributyrine	--	+
azetylcholine	+	+
acetyl- -methylcholine	+	--
benzoylcholine	--	+

[4]Area of concentration of hydrogen ions in which the enzyme concerned has the maximum activity.

investigations[5] and represents the basis of the highly sensitive methods of detection and determination. The principle of these methods is the comparison of the speeds of separation of the substrate in the enzyme in the presence of the warfare agent to be analyzed (inhibitor) and without it. The sensitivity of the method is determined among other things by the affinity of the enzyme with the inhibitor and the speed of the separation of the substrate. The degree and speed of inhibition of the enzyme can be affected by the choice of certain optimal conditions of the reaction. The use of the optimal substrate affects the state of the appearance of the analytical signal and consequently the sensitivity. Obtaining the optimal substrate is only possible if the mechanism of the catalytical effect of the enzyme is known.

29.2. Inhibition of Cholineesterase with Organophosphoric Compounds

It is apparent from Table 29.1 that the organophosphoric warfare agents inhibit a large number of vital enzymes in the organism. The effect is particularly marked for the cholineesterase while as we know the inhibition of the acetylcholineesterase determines particularly the most important features of the poisoning picture. The mechanism of the catalytical effect and the inhibition of this enzyme which is so important for the nerve function were elucidated to a great extent using the organophosphoric inhibitors as "chemical instruments".

Cholineesterase are all hydrolases (esterases) which give off cholineesters, and which are inhibited by physostigmine in a concentration of 10-5 M. These cholineesterases are subdivided into two groups, whose distinguishing features are indicated in Table 29.2.

The hydrolysis of the substrate catalyzed by the enzyme can be characterized by the Michaelis-Menten equation:

The enzyme and substrate form first a complex (ES) from which the products of the reation come. In the case of hydrolysis of acetylcholine by AChE acetylized enzyme and choline are formed; the acetylized enzyme is very quickly hydrolyzed with formation of acetic acid and enzyme in the original state.

The catalytical effect of the AChE is based on the structure of the enzyme protein. Through the folding of the protein the amino acids from remote areas of the chain come close and form the "active center". This center contains 2 "active points". One of them ("anion point of action") is responsible for fixing the substrate and to a great extent for the specificity of the enzyme; the other "esterase point of action" causes the separation of the substrate.

The following ideas have been put forward on the chemical nature of the points of action and their mechanisms: the carrier of the negative charge in the anion action point is probably the carboxyl anion of an amino dicarbonic acid (aspartic or glutamic acid). The binding of the substrate is accomplished by means of Coulomb forces between this group and the positively charged quarternary group of the acetylcholine. Moreover the van-der-Waals forces on the methyl groups and the methylene group of the substrate as well as weak covalent bonds between the electrophile carbon atom of the carbonyl group and the basic groups of the esterase point of action contribute also to fixing the substrate. In this way the substrate molecule is oriented in such a way that the carbonyl

[5] in vitro = in the test tube. Biochemical reactions which are not carried out in the living organism.

group approaches the esterase point of action at a distance of about 4-5 A from the anion action site and favorable conditions are created for the nucleophile attacks of the activated hydroxyl groups of the esterase action site on the carbonyl group.

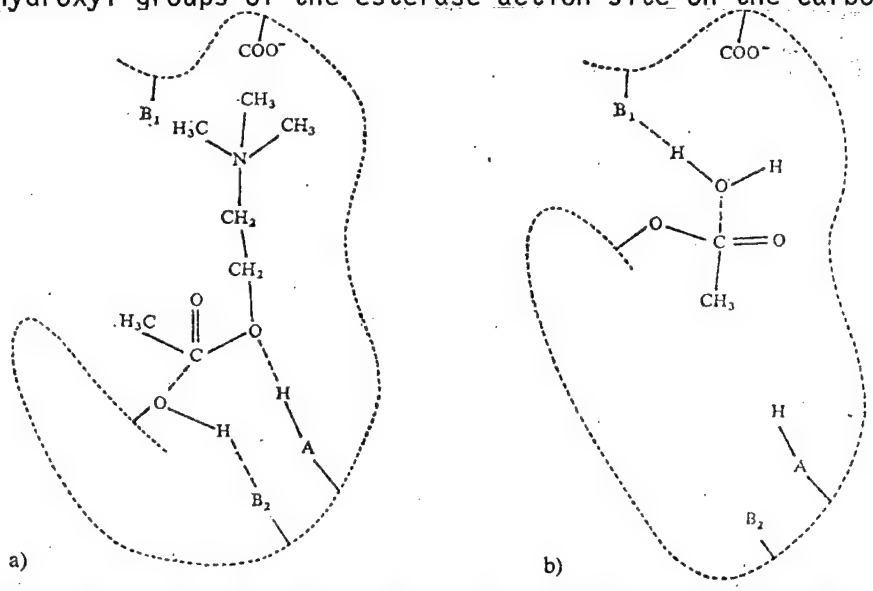
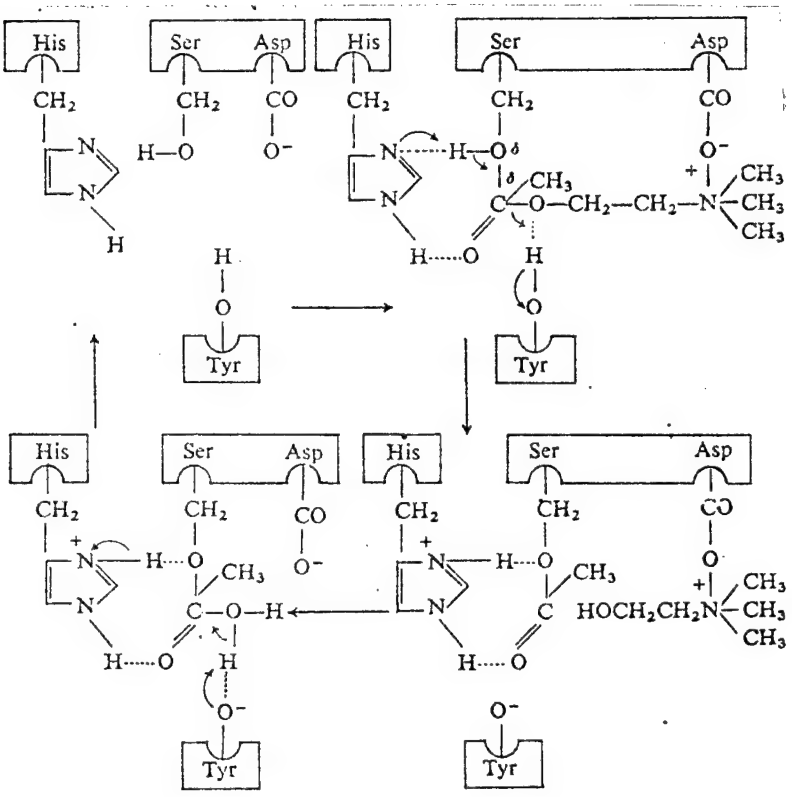


Figure 29.1: Representation of the active center of the acetylcholineesterase according to Krupka [121]. B1 and B2 are the basic groups; AH is the acid group; OH and COO- are serin hydroxy group and anion action site.

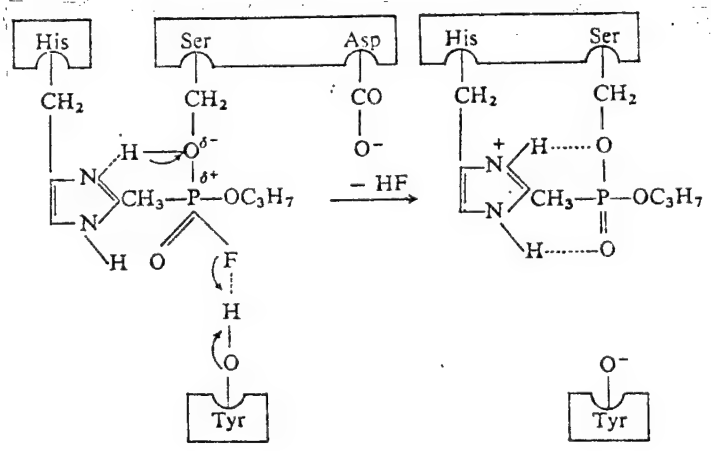
(a) enzyme substrate complex (ES) for acetylcholineesterase
(b) acetylized enzyme (EAc)

In the esterase action site we find an acid and basic acid group whose existence was first derived from the reversible loss of activity on both sides of the pH optimum. In the basic group we have the nucleophile hydroxyl group of serin. Since serin under physiologic conditions can neither catalyze ester hydrolysis nor react with alkylphosphates, it is assumed that an activation takes place through the neighboring amino acid residues. The pKa value of the basic action site was obtained as 5.8 to 7. The imidazol ring in histidine is the only protein group with a pKa (5.6-7.1) in this range. It is therefore assumed that the doubly bound nitrogen atom of imidazol takes a proton from the serin hydroxyl group and the anion formed attacks the substrate in a nucleophile manner. The acetylzed serin formed is brought by a change of conformation into the neighborhood of a second imidazol group, which takes a proton from water and thus causes the hydrolysis of the acetylzed serin. The acid active group in which we are dealing presumably with the hydroxyl group of tyrosine forms first a hydrogen bridge to the alcohol oxygen and causes in the next stage the separation of the choline residue. A simple scheme for the described mechanism of substrate separation was given by Krupka [121].

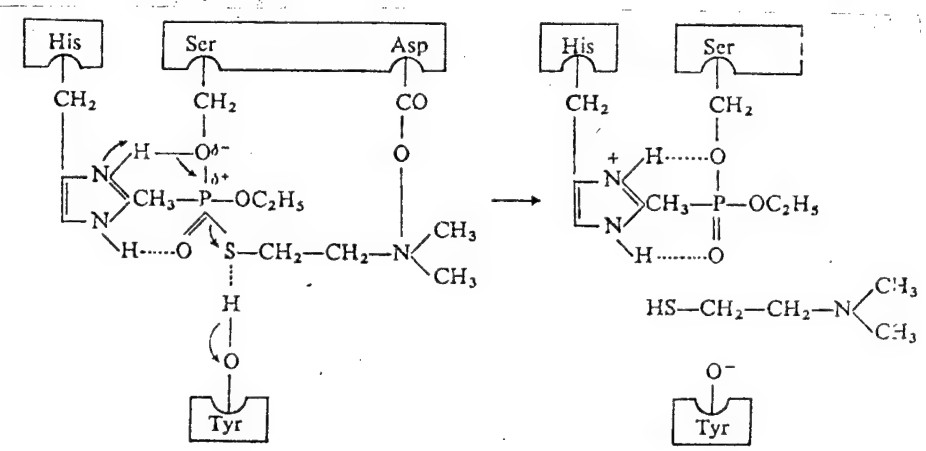
It should be added that these ideas of the enzyme catalyzed hydrolysis of choline ester are not final and for example a further scheme was developed for the mechanism [295] in which the acid active group causes first the splitting of the choline as proton donor and then in the anion form acts as proton acceptor causing the hydrolysis of the acetylized serin.



The irreversible inhibition of AChE through the organophosphoric warfare agent is based on a phosphorylation which corresponds in principle to the acetylization of the enzyme in the substrate hydrolysis. But unlike the acetylized enzyme the phosphorylized enzyme under physiological conditions is hydrolyzed only extremely slowly. Only with a stronger nucleophile reagent (reactivators) the phosphoric acid ester is separated more quickly from the enzyme. The phosphorylation of AChE through sarin can be described as follows.



The extraordinarily high inhibiting effect of phosphorylcholine and phosphorylthiocholine compounds ("Tammeline esters") shows that the affinity of an inhibitor to an enzyme and thus its inhibiting effect just like for the specific substrate can be increased by introducing a cation group taking into account the distance between anion and esterase active site (2 CH2 groups).



The phosphorylized enzyme is now transformed with a secondary reaction taking place with different speeds into a stage which can no longer be reactivated ("aging"). Since in an AChE aged and inhibited by sarin isopropyl alcohol was detected, this aging is due most probably to a dealkylation. In the case of the AChE phosphorylized by soman this reaction is completed already after 2 to 5 minutes.

The organophosphoric inhibitors are competitive inhibitors and as is apparent from the previous considerations interact with at least one of the same active sites of the enzyme as the substrate. The result is that in the presence of substrates the speed of the reaction of the inhibitor with the enzyme decreases with the increasing concentration of substrate. Therefore it is necessary in determinations of the inhibitor first to incubate the enzyme with the inhibitor and then to add the substrate to obtain the residual activity. Another factor very important for analytical methods is the different speed of the reaction of the individual organophosphoric inhibitors with the enzyme which must be taken into consideration when choosing the incubation time. Dialkylaminoalkylfluorophosphonates react very quickly with the enzyme, O-alkyl-methylfluorphosphonate (sarin, soman) only a little slower, the O-alkyl-S-(N,N-dialkylaminoalkyl)-alkylthiolphosphate and phosphonates (V-substances) on the other hand are much slower. The relations between the resistance to hydrolysis of the compounds and the speed of the inhibiting reaction are apparent. This is understandable since the catalyzed hydrolysis of the organophosphoric compound can be considered as model reaction for the phosphorylation of the enzyme. Instead of the hydroxide ion in the SN2 reaction the nucleophile center appears on the enzyme. For the nucleophile attack on the enzyme on the atom of phosphorus the differences in the charge distribution between the atom of phosphorus and the different neighboring atoms (F,S,O) act in the same way as in the hydrolysis on the reaction compatibility with the nucleophile reagent. Since the reaction of the organophosphoric inhibitors with the enzyme takes place much more quickly than with the hydroxide ion, it is possible to assume for the secondary reaction favorable fixing of the active sites of the enzyme.

The above considerations on the substrate separation and inhibition of the AChE can be transferred in many respects to the nonspecific cholineesterases (ChE). Of this group of esterases highly purified preparations have not yet been produced as for the AChE, therefore the properties of the structure are not known in such detail. For example the existence of an anion active site in the ChE is disputed, and one tends to the opinion that the fixing of the substrate in the ChE is accomplished mainly by the van-der-Waals forces. The differences existing undoubtedly in the structure of the active centers are apparent also from the different substrate specificity (see Table 29.2) and from the different inhibition capacity by selective inhibitors. Thus O-ethyl-S-(N,N-dimethylaminoethyl)-methylthiophosphonate is a selective inhibitor of AChE, whereas O,O-diethyl-S-(N,N-dimethylaminoethyl)-thiophosphate inhibits the ChE more strongly /122/.

More recent investigations /165/ with selected organophosphoric inhibitors show that in the inhibition process of choline esterases the hydrophobicsorption in the hydrophobic regions in the active centers of the enzyme play an important and sometimes a decisive role. Through the interaction of these hydrophobic regions with the alkyl radicals of the inhibitors for example it is possible to explain also the extraordinarily high anticholineesterase activity of pinacol esters (soman).since it was established that both in the AChE and in the ChE in the neighborhood of the anion active site there must be a hydrophobic region which is the most complementary for radicals with highly branched butyl groups. Moreover it was established that immediately around the anion active sites a hydropholic region must exist to which greater importance must be assigned for the substrate fixing in the ChE than in the AChE. In the neighborhood of the esterase active site for the AChE another hydrophobic region is suspected and two more for the ChE. Probably these differences determine significantly the different properties of both cholineesterases.

29.2.1. Determination of the Inhibiting Effect

The characterization of the inhibiting effect is carried out by determining the concentration of inhibitor which inhibits the enzyme up to 50 percent under certain conditions. This concentration is designated as I50; in most cases it is indicated in the form of its negative logarithms as pI50.

$$pI50 = -\log I50$$

These values are typical constants of the substances (compare Table 29.3.). The assumption for the comparability of the values is their determination under exactly defined conditions, that is under certain temperature, time of incubation and reaction, concentration of enzyme and substrate and definite pH values. For example in the electrometric method, the enzyme is incubated for 30 minutes at 25 degrees C; the initial pH value is 8.0 and the reaction is allowed to proceed for 60 minutes with a substrate concentration of 7.3 by 10-3 moles ACh/l /123/. To obtain the I50 the activity is measured with different inhibitor concentrations. The diagram is prepared in which the measured ratio of the inhibited enzyme to the noninhibited enzyme (in percentage of inhibition) is plotted against the negative logarithm of the inhibitor concentration. An example of this procedure may be found in Figuare 29.1. The informative content of the pI50 values is limited by different secondary reactions which take place as a function of the properties of the inhibitor and the enzyme and the experimental conditions. Such secondary reactions which compete with the enzyme inhibition are the spontaneous hydrolysis, the hydrolysis of the inhibitor through the phosphorylphosphatases contained in the enzyme, the adsorption

on the wall of the glass containers with very dilute solution and the reaction with other active groups of the proteins present, moreover with weak inhibitors the inhibition and reactivation can take place simultaneously.

Table 29.3: pI50 Values and Speed Constants of the Second Order for the Inhibition of Cholineesterases and the Alkaline Hydrolysis

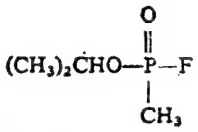
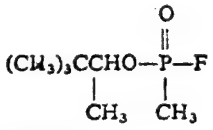
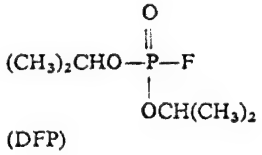
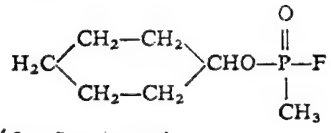
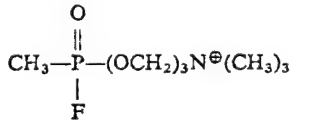
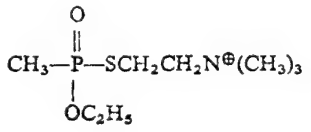
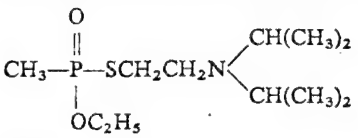
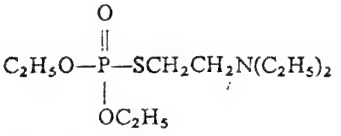
Inhibitor	pI_{50}		k_2 bei 25 °C $l \cdot mol^{-1} \cdot min^{-1}$		k_2 OH^- bei 25 °C $l \cdot mol^{-1} \cdot min^{-1}$
	AChE	BuChE	AChE	BuChE	
 (Sarin)	8,9	8,4	$1,4 \cdot 10^7$	$1,7 \cdot 10^7$	1560
 (Soman)	9,2	8,6	$6 \cdot 10^7$	$2,1 \cdot 10^7$	83,3[5])
 (DFP)	6,6	7,8	$4,8 \cdot 10^4$	$1,5 \cdot 10^7$	49,8
 (Cyclosarin)	10,1[1])		$3,3 \cdot 10^8$	$1,6 \cdot 10^7$	
	11,0	8,4		$1,1 \cdot 10^7$ [2])	18300
	9,1	7,9		$1,2 \cdot 10^5$	12

Table 29.3. (continued)

Inhibitor	pI_{50}		k_2 bei 25 °C $l \cdot mol^{-1} \cdot min^{-1}$		k_2 OH^- bei 25 °C $l \cdot mol^{-1} \cdot min^{-1}$
	AChE	BuChE	AChE	BuChE	
 (VX)				$2{,}3 \cdot 10^6$	1,2[3])
 (Amiton)	7,9[4])	8,3	$2{,}5 \cdot 10^6$	$3 \cdot 10^7$	0,8

1) cattle erythrocyteesterase.
2) in the presence of substrate in a concentration of 1.87 by 10-2 mole/l.
3) in seawater.
4) at 37.5 degrees C.
5) at 20 degrees C.

Some authors consider the indication of the bimolecular speed constant k2 of the reaction between inhibitor and enzyme

$$EH + P \,.\, X \rightarrow E \,.\, P + HX$$

as specific and particularly for slow reacting inhibitors as depending on the properties of the substance (Table 29.3. gives some of the values known from the literature for k2), but the I50- or pI50- values can be considered as more informative with regard to the toxicity of the inhibitors.

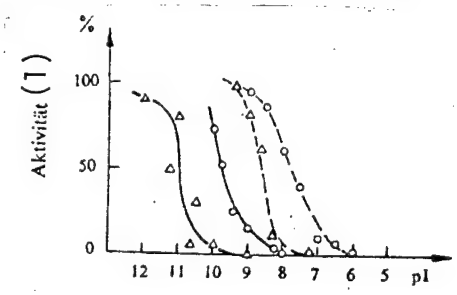


Figure 29.2: Diagrams to obtain the pI50- value
_____AChE; -------ChE; △ methylfluorophosphoryl-choline; ○ methylethoxyphosphorylcholine (V-substance) (taken from Tammelin, L.E.; Ark. Kem. 12 (1958),S.287).
(1) activity

This is proved by the fact that the V-substances reacting slowly with the enzyme have approximately an equal toxicity as the very quickly reacting methylfluorophosphorylcholine.

Between the I50 value and the bimolecular speed constants k2 the following relation exists.

$$k2 = \frac{0.695}{I50 \,.\, t}$$

29.2.2. Determination of the Cholineesterase Activity

Whereas the purpose of the AChE, the fast disintegration of the acetylcholine formed in the transmission of nerve pulses is fixed, the physiological function of the ChE has not yet been clarified completely. In case of poisoning with organophosphoric compounds a decrease of the ChE activity can be established but one which is not always proportional to the seriousness of the clinical symptoms. Thus a decrease of the activity by 85 percent of the initial level was established without the occurrence of symptoms; on the other hand in poisonings by inhalation strong symptoms can appear without considerable change in the activity. But in general the determination of the activity of the ChE in the serum offers a satisfactory possibility of diagnosing poisoning with organophosphoryls. A decrease to 40 percent of the normal value can be evaluated as a beginning of damage, if other factors which reduce the activity, such as hepatitis, anemia or various infectious diseases can be excluded. A more reliable, but less sensitive indicator of the poisoning with organophosphoryls is the determination of the AChE activity. It is therefore less suitable for the prophylactic examination of people working with organophosphoric compounds, the more so as the ChE activity is more easily measured because of the greater selection of substrates.

To determine the activity of the AChE in the blood of men and mammals the red blood corpuscles (erythrocytes) were hemolized; the ChE is determined in serum. In case of determination in full blood a differentiation can be achieved by choosing specific substrates, such as acetyl-beta-methylcholines for AChE and butyrylcholine for ChE, or by using certain substrate concentrations. The last method is based on the already known inhibition of the ChE by higher substrate concentrations. Thus for a concentration of 10-3M acetylchloline the AChE is recorded mainly, for 10-1M the ChE. Naturally each time the other enzyme is included to a certain extent (about 1/5).

The determination takes place either by obtaining the speed of the enzymatic substrate separation reaction ("kinetic method") or by determining the final products or the nonconverted substrate ("final value method").

To characterize the enzyme activity according to the method of determination various mass numbers are used. For the classical manometric method the amount of carbonic acid released is the measure of the activity. Ammon [12] defines as "cholineesterase unit" the amount of enzyme which in 1 hour at 37 degrees C and pH 7.4 can separate 10-6M acetylcholine. This amount of acetylcholine corresponds also to 10-6 M carbonic acid (=22.4 mm3). To evaluate the activity obtained in the blood of a control subject the knowledge of the normal values and their spread is important. For a test with 0.05 ml serum for the manometric maintaining the above-mentioned conditions in normal value of 7 to 10 cholineesterase units is indicated [12]. In the colorimetric method by determination of the nonconverted acetylcholine [13] the cholineesterase unit taken is the number of micromols of acetylcholine which are hydrolized by 1 ml serum in 1 hour at 37 degrees C. This corresponds to 20 times the Ammon unit. The normal value indicated is 130 to 310 units.

In the electrometric method the cholineesterase activity is expressed in the drop of pH after 60 minutes reaction time. The normal value at 25 degrees C and using 0.2 ml serum is a Δ pH value of 0.70 to 1.05 [14].

To determine the cholineesterase activity a large number of methods have been established. The most reliable values are given by the methods based on the determination of the product produced, in which a linear relation exists between the enzyme activity and the measured value. Although it is complicated to implement, in this respect the manometric method has not yet been surpassed. The electrometric methods are simple to implement and give very exact values particularly when using an autotitrator by keeping constant the pH value affecting the enzyme activity. We give below a survey of the different methods of determining the activity. For a detailed description the manometric and electrometric methods were selected for the above-mentioned reasons.

I. Determination of the acid formed in the separation of the carboxylcholines.

1. Manometric methods [125, 126, 127]

They are based on the measurement of the CO2 volume which is released by the acetic acid formed in the separation of acetylcholine from the bicarbonate buffer. It is established with the known Warburg equipment. A new warburg vessel suitable particularly for the measurement of inhibition of ChE has been described by Adie [131].

2. Titrimetric method

The acid released is titrated after adding a suitable indicator (bromothymol blue, phenol red) or by control with a pH measurement equipment in 5 minute intervals for a period of 1 hour with sodium hydroxide every time the same pH value [132].

The titration with an autotitrator through which the pH value is kept automatically constant so that the enzyme activity undergoes no change during the measurement is very advantageous [129].

3. Electrometric method (ΔpH methods)

For these methods the change in the pH caused by the acid after a certain period is measured with a pH measuring equipment. A special buffer developed by Michel [130] with an initial pH value of 8.0 is used, whose buffer properties guarantee a linear relation between the enzyme activity and the variation of the pH. Another advantage of the method lies in the possibility of studying parallelly a very large number of specimens. The methods whose results appear as a pH value can be calibrated by adding standard acid to the buffer enzyme mixture per micromol separated acetylcholine. The drop of the pH value is measured for certain amounts of acid, and on the basis of these values a calibration curve is established from which it is possible to obtain the amount of substrate separated.

4. Colorimetric methods with pH indicator

The enzyme specimen is treated with substrate and a suitable indicator, therafter we determine the time up to the identity of the color with that of a standard solution. The pH value of the standard solution produced by adding suitable amounts of acid is conveniently in the area of the most sensitive change in color of the indicator, for example for bromothymol blue at pH 6.8 to 7.2. The measurement of time can be carried out by visual colorimetric or photometric determination of the identity of color. Other suitable indicators are phenol red and M-nitrophenol.

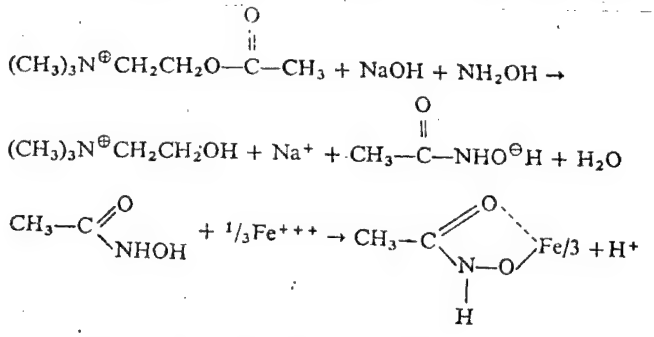
5. Methods with substrate indicator papers

These papers developed by Herzfeld and Stumpf [133] by modification of the indicator method are used for the clinical rapid determination of AChE and ChE and are produced commercially with acetylcholine as substrate and bromothymol blue under the designation "acholest" [134] and "biophan C" [135]. The paper described by Matousek and Cerman [136] with butyrylcholine as substrate gives faster indications and is specific for ChE. After incubation of the papers on an object carrier with a drop of serum (0.05 ml) the time taken for the color to be identical to a standard (pH 6.47) is measured.

In a patent of the Merck Company [166] a paper impregnated with an alcoholic solution of 15 percent acetylcholinechloride and 0.1 percent indicator mixture (phenol red and naphtholphthalein 1:1) is described. After the addition in drops of the serum (0.04 ml per cm2) within 6 to 8 minutes in amount of substrate differing as a function of the ChE activity is separated, and in the pH range of 6 to 8 the different shades of the indicator are adjusted which can be compared with a color scale.

II. Determination of the nonseparated acetylcholines

After 30 or 60 minutes reaction time by adding alkaline hydroxylaminehydrochloride solution the excess acetylcholine into hydroxamiacetic acid, which forms at pH 1.0 to 1.4 with iron(III)-chloride a reddishbrown internal complex salt with an absorption maximum at 520 nm.



The method established originally by Hestrin [137] gives especially according to the procedure described by Pilz [138] very reliable values and is frequently used. The determination of the excess acetylcholines can also be carried out according to the principle of the Schoenemann reaction (compare section 28.1) [139].

III. Determination of the efficient products of carboxylthiocholines

Carboxylthiocholines are separated more quickly than cholineesters and because of the manifold possibilities of determining the efficient product thiocholine they are used extensively. In the simplest case the removal of the substrate is determined by UV spectral photometric measurement (maxima of the acetylthiocholines at 226 or butyryl-cholines at 250 nm).

1. Colorimetric determination of thiocholines

With sodium nitroprusside thiocholine produces a color (λ max 520 nm). Another photometric method is based on the measurement of the yellow color which arises in the reaction of thiocholine with 5,5-dithio-bis-(2-nitrobenzoic acid) [140]. This method is very convenient in its procedure and requires only a short time.

2. Electrochemical determination

A modern method [142] is based on the depolarisation of a platinum electrode through the thiocholine which is oxidized on the anode into diosulfide. The work is carried out in the tris-buffer at pH 7.4 with substrate concentrations of 2.10-3 M butyryl-thiocholine iodide for ChE and the same concentration of acetylthiocholineiodide for AChE. One calomel and two platinum electrodes are dipped into the substrate buffer solution. On the platinum electrodes a constant current of 25 μA is applied. After adding the enzyme solution, the change of voltage between the platinum electrode and the kalomel electrode is plotted on an automatic recording instrument against the time. From the curve obtained the slope $\Delta E/\Delta t$ (in millivolts per second) is determined and from the calibration curve which was recorded with known enzyme activities it is possible to obtain the activity of the specimen enzyme.

The method is applicable to determine the enzyme activities and the concentration of substrates and inhibitors. The presence of the iodide ion in the solution to form the initial potential ($2J^{\ominus} \rightarrow J2 + 2e-$) is indispensable and is assured by the use of iodide as acetylthiocholine.

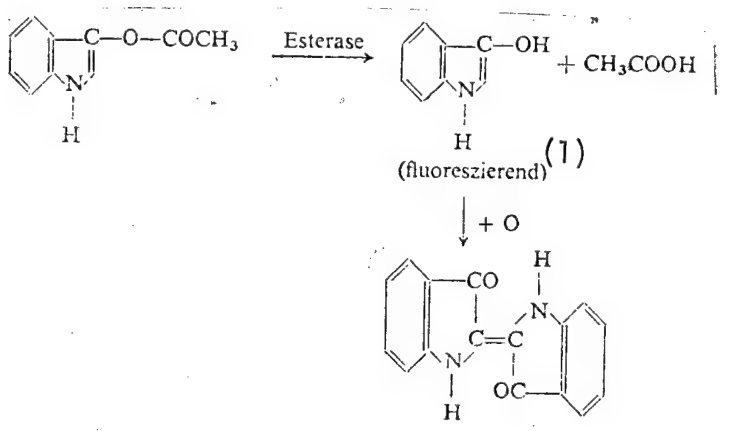
Since acetylthiocholine is polarographically inactive, while thiocholine on the other hand shows an anodic stage typical for -SH groups, for the growth of the anodic stage per unity of time we may derive a measure of the activity of the cholineesterase [143]. The polarographic determination is less time consuming.

The determination of the activity by means of a liquid membrane electrode selective for acetylcholine ions is very elegant [167]. The ratio of selectivity of the electrodes against choline is 15 to 1. The potential measured against a kalomel shows the linearity of the curve E = f (lg a) up to 10-5 M acetylcholine. The time up to the change of the concentration of the substrate from 1.95 by 10-3 M to 1.85 by 10-3 M is used for the calculation. The unit used is the amount of enzyme which hydrolyzes at pH 8.0 within 1 minute 1 by 10-5 mol substrate.

IV. Determination with chromogenic and fluorogenic substrates.[6]

The use of substrates which in the separation of the carboxyl group a colored or fluorescent product is very advantageous for the histochemical location and for the colorimetric and fluorimetric determination of the activity of esterases. Some of these substrates are separated specifically from certain esterases. For histochemical studies the most commonly used are indoxylacetate and a few halogen substituted derivatives of indoxylacetate, mainly the 5-bromo-4-chloroindoxyl. The evaluation or measurement is carried out either through the fluorescnet indoxyl [157] or colorimetrically after oxidation to indigo or indigo derivatives [144].

[6] substrate which gives for an enzymatic separation a colored or fluorescent product.



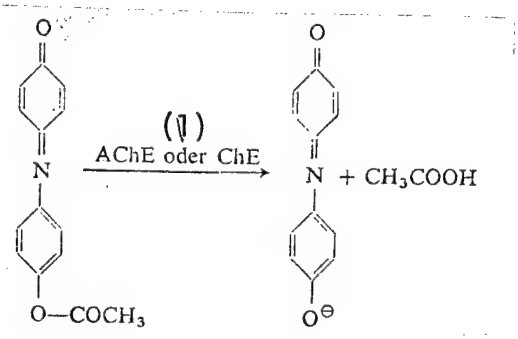
Key: 1) fluorescent

Guilbault [168] recommended instead of indoxylacetate the N-methylindoxylacetate which is separated more quickly and whose fluorescent efficient product N-methyl-indoxyl has a greater stability against the oxidation of air than the nonfluorescent indigo green, which makes the measurement of fluorescence more exact.

The separation of resorufinacetate by ChE at pH 7.4, by phosphatase and α and β chymotrypsin gives the highly fluorescent resorufin of which 10-8 mol can still be detected fluorometrically [158].

The fluorescence of the naphthol formed in the enzymatic separation of α and β naphthylacetate is also used for the very sensitive measurement of enzyme activities. Likewise in the hydrolysis of methylumbelliferonacetate by ChE in phosphate buffer of pH 8.0 the highly fluorescent methylumbelliferon is obtained [171].

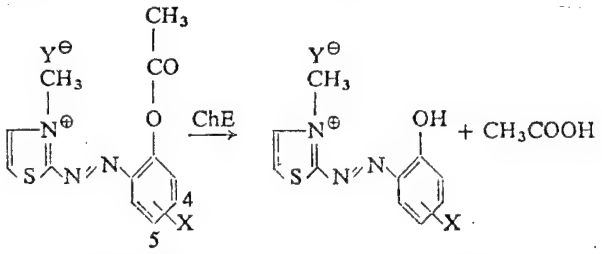
Some methods are based on the measurement of the separation of indophenylacetate at pH 8.0 [145]; the blue color of the indophenol produced is measured at 625 nm. The method gives exact and reproducible values at 25 to 150 cholineesterase units according to Ammon.



Key: (1) or

The drawback of this substrate is that the phenolate ion formed is dissociated completely only for pH values over 9.5 and thus in the colorimetry the maximum sensitivity cannot be achieved for the pH value of 8.0 optimum for the substrate separation. On the other hand the use of 2,6-dichloroindophenylacetate is advantageous because in this case the phenolate ion is dissociated totally already at pH 6.5 and the separation of this substrate moreover takes place at a speed about

three times higher [169]. The measurement of the color takes place at 610 nm. Other very suitable chromogenic substrates are some of the 1-(2-thiazolylazo)-2-acetoxybenzol derivatives [170]:



X = 5-CH3; Y=(CH3)2SO4 yellow → blue (585 nm)
X = 4-N(CH3)2; Y = J blue → red (484 nm)

The hydrolysis of substrates of this type takes place with optimal speed in the phosphate buffer at pH 7.0.

29.2.2.1. Determination of the Cholineesterase Activity in the Serum with the Warburg Apparatus [125]

Reagents:

A. Sodiumbicarbonate/Ringer solution

The following are mixed: 30 ml 1.26 percent NaHCO3 solution, 100 ml 0.9 percent NaCl solution, 2 ml 1.2 percent KCL solution and 2 ml 1.76 percent CaCl2 by 6 H2O solution.

B. Substrate solution

50 ml acetylcholinechloride are dissolved in 10 ml solution A (prepared fresh every day!)

Procedure:

The work is carried out with a measurement volume of 2 ml; the measurement temperature is 37 degrees C; the gas phase contains 5 volume percent CO 2 in nitrogen. In the main chamber of the Warburg vessel 1.5 ml substrate solution are placed, in the attachment 0.5 ml of the serum diluted 1 to 50 with solution A. After 10 minutes tempering the manometer cocks are closed and the serum is dipped into the main chamber. At intervals of each time 10 minutes the manometer readings are taken for 1 hour and the results are noted. For each determination a control value is taken to measure the spontaneous hydrolysis of the substrate, containing instead of serum solution A.

Calculation:

The pressure increase read (in millimeters Brodie solution) is corrected each time by the amount of the control value; the corrected values are multiplied by the vessel constant. The millimeters of carbon dioxide are plotted against the time; after graphic averaging the 60 minute value is divided by 22.4 µl and we obtain the micromol of acetic

acid (= micromols of the acetylcholine separated). Since 0.01 ml serum was introduced, to calculate in cholineesterase units one must multiply by 100. The normal value is 150 ro 200 units.

Remark:

One may obtain further information in the relevant literature on the general procedure with Warburg equipment which needs some practice [164].

29.2.2.2. Determination of Cholineesterase Activity According to the ΔpH Method [124]

Reagents:

A. Buffer solution (Michel buffer)

1.2371 g sodiumveronal[7] (0.006 m), 0.1361 g potassiumdihydrogenphosphate (0.001 m) and 17.535 g sodiumchloride (0.3 m) are dissolved in 900 ml water free from carbon dioxide and treated with 10 ml 0.1 normal hydrochloric acid. After filling to 1 l the pH value is measured at 25 degrees C and should be about 8.00. If necessary it is adjusted exactly with 0.1 normal hydrochloric acid. Two drops of toluene are added for preservation.

B. Substrate

3 g acetylcholinebromide are dissolved in 100 ml water and treated with two drops toluene. The solution is kept in the refrigerator.

Procedure:

10 ml distilled water, 0.2 ml serum and 10 ml buffer (A) are pipeted into a 50 ml Erlenmeyer flask. This mixture is tempered for 10 minutes in the thermostat at 25 degrees C and then we determine with a glass electrode measurement chain and a pH measurement device the pH value exactly within 0.01 pH. Then 2 ml substrate (B) are added, we mix quickly and note the time. After exactly 60 minutes the pH value is measured once again. When several specimens are processed, we work with short time intervals; the electrode is then dipped again each time 30 to 60 s before the measurement to temper the system.

Calculation:

pH1 - initial pH value
pH2 - final pH value
t1 - time of added substrate
t2 - time of reading the pH2
b - correction factor for spontaneous hydrolysis in accordance with pH2
F - correction factor of the deviations of the ΔpH/h

[7] sodium diethylbarbiturate acid.

Table 29.4. Correction Factors

pH_2	b	F
7,9	0,09	0,98
7,8	0,07	1,0
7,7	0,06	1,01
7,6	0,05	1,02
7,5	0,04	1,02
7,4	0,03	1,01
7,3	0,02	1,01
7,2	0,02	1,0
7,1	0,02	1,0
7,0	0,01	1,0
6,8	0,01	1,0
6,6	0,01	1,01
6,4	0,01	1,02
6,2	0,01	1,04
6,0	0,01	1,09

Remarks:

If the method for determining the activity of dry sera and enriched sera is used care must be taken that about 12 to 13 mg protein are contained in the sediment; this is needed to control the protein component for the buffer capacity of the system and therefore the comparability of the values. The method can be used to determine the I50 values and the inhibitor concentrations [96]. For this last purpose the serum buffer mixture is incubated for 30 minutes with the inhibitor solution before adding the substrate. A control experiment without an inhibitor is started parallelly. From the pH values measured after the reaction period the inhibition is calculated by the following formula:

$$\frac{(pH1 - pH2K) - (pH1 - pH2P)}{(pH1 - pH2K)} \cdot 100 = \text{percentage inhibition}$$

pH1 - initial pH value
pH2K - final pH value of control
pH2P - final pH value of the specimen.

The inhibitor concentration corresponding to the calculated inhibition is taken from a calibration curve established with known inhibitor concentrations. The work is carried out with standardized solutions of dry serum.

29.2.2.3. Photometric Determination of the Cholineesterase Activity [141].

This micromethod which can be carried out quickly is based on the reaction of the thiocholine formed in the enzymatic separation of the acetylthiocholine with 5,5-dithio-bis-(2-nitrobenzoic acid)(DTNB). The mechanism of this thiol color reaction is described in section 28.1.1.5.

Reagents:

A. DTNB buffer solution

25 mg DTNB, 1.66 g sodiumchloride, 62.5 ml 0.2 molar trisbuffer solution (=tris /hydroxymethyl/aminomethane) and 100 ml 0.1 normal hydrochloric acid are placed in a 250 ml measuring vessel and filled up with distilled water. The pH value of this buffer solution is 7.4 at 37 degrees C; it is stable for two weeks when kept in the refrigerator.

B. Substrate solution

0.52 g acetylthiocholineiodide are dissolved in 100 ml water (0.018 molar).

C. Quinidinesulfate, 0.5 percent aqueous solution or 0.5 percent DFP solution

Procedure:

Two test tubes of which one is used for the specimen, the other for the control tests are filled with 4 ml A and tempered for 5 minutes in water bath at 37 degrees C. To this end 0.02 ml is added for each and thereafter in the control experiment 1 ml C. 0.5 ml B is added to each of the two tubes, and mixed. Exactly 3 minutes after adding B the reaction in the specimen is stopped by adding 1 ml C and the extinction of the specimen and control determined immediately afterwards by photometric measurement at 412 nm..

Evaluation:

The measured difference of extinction of sample and control experiment is converted to the micromol of separated acetylthiocholine per 3 minutes and 1 ml serum. To this end a factor is used obtained from a calibration curve which was plotted with glutathion or another substance with known content of SH groups.

The normal values found were 7.8 to 16.6 µmol SH per 3 minutes and 1 ml for man and 5.8 to 13.8 for women.

29.2.3. Determination of Cholineesterase Inhibitors

All the methods mentioned for determining the activity are suitable when modified accordingly for the sensitive determination of the inhibitors, except for the manometric determination whose sensitivity is too low for trace analyses.

The difference in the procedure consists in the use of enzyme solutions of known content which are incubated for a certain time with the inhibitor. Thereafter the substrate is added and the residual activity of the enzyme determined by the usual method. The incubation time necessary to achieve a sufficient sensitivity depends mainly on the speed of the reaction of the inhibitor with the enzyme. The reaction of phosphorylthiocholines with the enzyme can be considered as complete after 2 hours. The advantage here is that these components are exposed only to a very slight extent to hydrolysis and hardly any secondary reactions. On the contrary for the methylfluorophosphorylcholines which react fast with the enzyme very short incubation times are used. For the fluorophosphonates of the sarin type in general 10 minutes of incubation are sufficient for sensitive recording, for VX and the insecticide organophosphates it is proper to incubate for 30 to 60 minutes.

Since the inhibition depends to an equal extent on the pH value as the substrate separation, it is necessary to maintain the optimum pH range for the corresponding enzyme during the incubation with the inhibitor. For VX and similar compounds which have a tertiary amino group in the molecule care should be taken that the inhibition effect depends on the degree of ionization of the compound. For pH values above 8.5 the component of nonionized compound increases and the inhibition decreases. Anotehr prerequisite for reproducible results is the use of enzyme preparations with standardized activity. The one used most commonly is lyophilized horse blood serum. Insofar as it is not available as a commercial product, it can be produced on the laboratory scale by lyophilization (freeze drying) of liquid serum by relatively simple means.

To this end the liquid serum is frozen in a glass dish and placed in a vacuum exsiccator; the latter is connected through glass tubes with the largest possible cross-section through a cooling trap with an oil vacuum pump. According to the vaccum, which must be at least 10-2 torr, the serum is dried completely after a few hours or days. Before the use for analytical purpose, the activity is determined by means of a standard method such as the electrometric pH method. To record the calibration curves and carry out the determinations then each time enzyme solutions of the same activity are prepared.

It is advantageous to use purified enzyme preparation from which a part of the protein ballast was removed by fractionated precipitation with ammoniumsulfate for different pH values [132, 147, 148]. The dry substances obtained after centrifuging of the deposits and after dialysis and lyophilization then have as compared with the normal serum an increased activity according to the degree of purification, lower buffer effect and lower tendency to denaturation.

The initial materials for AChE preparation include primarily mammal erythrocytes, but the production raises difficulties, because the AChE obviously adheres to the cell membrane. To separate the stromas, previously lysolecithin, pancreas extracts and the surface active polyoxyethylenesorbitanlaurate (=Tween 20). The best preparation described so far show however only the specific activity of 42 with low yield. It was possible to obtain the most strongly enriched preparations with a specific activity of 11,000 by chromatographic purification of extracts from the electrical organs of electroporous electricus (electric eel) [172]. Using electrochemical methods in which the blood dye does not interfere, it is possible to use with hemolyzed erythrocytes. The hemolysis takes place by treating the erythrocytes with a saponine solution.

The characterization of the activity of the enzyme preparations takes place in accordance with the nomenclature proposals of the International Union for Biochemistry (1964) as specific activity in enzyme units per milligram protein. The enzyme unit is the amount of enzyme which converts in 1 minute 1 mol substrate (here acetylcholine) under the conditions indicated (here mostly pH 7 to 7.5 and 37 - 38 degrees C). To convert the enzyme dry substance to protein a protein determination must be carried out by the Kjeldahl method.

It is basically necessary before using the enzymatic method on specimen materials to establish a calibration curve or calibration table with the pure inhibitor substance. Maintaining exactly the experimental conditions, including also the composition of the specimen solution corresponding to the later application, the measurement values are obtained, mostly after conversion to percentage inhibition, plotted against the concentration or its negative logarithm.

The method corresponds to the determination of the I50 or pI50 values. In the measurement as far as possible we should work in the region of a 40 to 60 percent inhibition of the enzyme, in no case outside the 20 to 80 percent inhibition. The organic insecticide thiophosphoric compounds are oxidized in vivo to their oxygen analogs or to their sulfoxides and sulfones, which are strong cholineesterase inhibitors. Before the enzymatic determination it is therefore oxidized in vitro; this takes place through the effect of bromine water of N-bromosuccinimide, hydrogen peroxide/acetic acid or silver oxide. The excess bromine is removed by adding some phenol water.

A survey of the most common methods used to determine cholineesterase inhibiting organic phosphoric compounds is given as follows:

I. Methods with Carboxylcholine as Substrate

The visual colormetric method of the measurement of the acetic or butyric acid formed with bromothymol blue is very sensitive /149/. The time elapsing until the color is the same as that of the standard is measured. The inhibition is calculated from:

$$\text{percentage inhibition} = 100 - \frac{tK \cdot 100}{tp};$$

tK - working time of the control specimen
tp - working time of the specimen.

This method is excellent for the field analysis determination of organophosphoric warfare agents because of the small demands which are imposed on the equipment. The standard (reference color) is produced by mixing in a test tube serum solution, the solution to be studied, bromothymol blue and a certain amount of acetic acid. Two other test tubes are also coated with serum solution; in one of them the solution to be studied is poured, in the other unpoisoned water (control specimen). After the incubation time acetylcholine solution is pipeted quickly one after the other into the two tubes and the time noted. After a few minutes the control specimen reaches the color of the standard. The specimen with the solution to be studied reaches in case of an inhibition of the enzyme through the poison present this yellowish green color only after a correspondingly longer time. From the comparisons of these two periods it is possible to calculate the inhibition according to the formula and to obtain from the calibration curves the corresponding inhibitor concentration.

The titrimetric determination of acetic acid from acetylcholine with phenol red as indicator was used to determine 0.1 γg tabun and sarin per milliliter [150]. The ΔpH method is used very often [156]. To follow very fast inhibition processes, for example in the dialkylaminoalkyl-methylfluorophosphonates, the pH stat method in which the pH value is kept constant with the autotitrater is most suitable. An advantage of this method is that the addition of buffer ions which affect to a certain extent the enzymatic activity, is not needed.

The substrate indicator papers are suitable preferably for the field analysis of warfare agents containing phosphorus [136]. The advantages reside in the very simple manipulation, the low cost, the quick execution, the low requirements in reagent and the large temperature range in which the work can be carried out. Another improvement is represented by papers which contain besides the substrate and the indicator lyophilized serum [151]. Using purified enzymes and dry storage they can be kept for a long time.

In a study by the Dutch Research Center for Defense [173] paper strips are described which are impregnated with serum cholineesterase. According to the effect of the water to be studied, a solution of 2,6-dichloroindophenylacetate is added in drops. If the specimen is not poisoned, the papers quickly assume a blue color; in case inhibitors are present they remain colorless. The limiting concentration indicated is 0.1 μg/ml.

An excellent method for the parallel determination of a large number of specimens is a modified agar diffusion method of Sandi and Wight [152]. Holes are pierced in a smooth layer of agar gel containing enzyme and indicator (bromothymol blue) applied on glass plates and the specimen solution is introduced in them. Diffusion is allowed for a certain time, as far as possible overnight, then acetylcholine solution is poured on it and we obtain on a yellow background, blue circles whose diameter is proportional to the inhibitor concentration of the specimen and allows the recording of a calibration curve. Parathion can be determined by this method up to a concentration of 0.03 μg/ml.

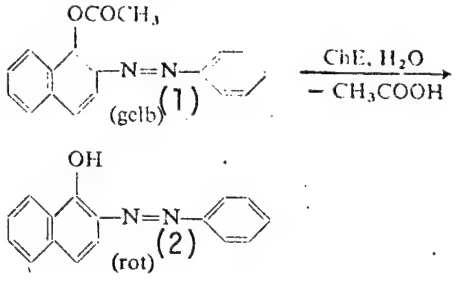
Using the selective electrode already mentioned in section 29.2.2. for acetylcholine ions it is possible to determine paraoxon and amiton in concentrations from 0.01 μg/ml [174]. After an incubation time has elapsed between enzyme and poison acetylcholine is added in a concentration of 2 by 10-3 M and by means of the electrode we find the time during which the substrate concentration has dropped from 1.95 by 10-3 M to 1.85 by 10-3 M. This time depends on the degree of the inhibition of the ChE and is thus a measure of the concentration of the inhibitor.

II. Methods with Carboxylthiocholine as Substrate

The already mentioned electrochemical method [142] which is based on the measurement of the depolarization rate of a platinum electrode was used to determine the organophosphoric cholineesterase inhibitors /153/ while for example for an incubation time of 10 minutes 2 by 10-4 μg sarin or 1 by 10-2 g systox per milliliter could be determined.

III. Methods with Chromogenic and Fluorogenic Substrates

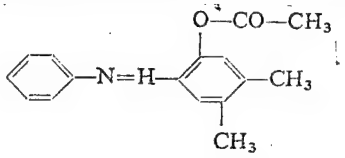
A chromogenic substrate is 2-azobenzol-1-naphthylacetate which is used in association with horse blood serum to determine sarin [159] and gives in the enzymatic hydrolysis at pH 7.4 to 7.8 the red 2-azobenzol-1-naphthol:



Key: (1) yellow; (2) red

After the reaction time has elapsed the enzymatic reaction is stopped by adding acetonic hydrochloric acid and the colored product is determined photometrically.

In a publication by Stachlewska-Wroblowa [175] it is indicated that of a series of acetylester derivatives of the hydroxyazobenzols the following compound is very suitable as a chromatogenic substrate in the enzymatic determination of soman with ChE:



For an incubation time of 10 minutes it is possible to determine 5 by 10-7 mg soman/ml.

Some simple field devices for the detection of nerve damaging chemical warfare agents are based on the splitting of 6-bromo-2-naphthylacetate through AChE to acetic acid and 6-bromo-2-naphthol and its combination with a stabilized diazonium salt into a blue azodye [161].

A very interesting enzymatic method for the detection of vapors and aerosols or organophosphoric warfare agents of the type G and V is described in an American patent [176]. By means of a double enzyme substrate system it is achieved that in the presence of an inhibitor a color is formed. In a mixture of reagents cattle plasma albumin hydrolyzes 3', 5'-dichlorindophenylacetate into blue indophenol; horse serum ChE hydrolyses acetylthiocholine into thiocholine. The latter reduces the indophenol to a colorless substance. Whereas in the absence of an inhibitor the reagent remains colorless. In the presence of poisons it becomes gradually blue because of the slowing down or the total suppression of the acetylcholine hydrolysis. The variation of extinction in the unity of time is the measure of the concentration of inhibitors.

A fluorimetric method for trace determination of organophosphoric insecticides [177] using N-methylindoxylacetate and ChE is interesting because it was shown that the ChE from different sources (human serum, liver, insect heads) is inhibited very differently by the corresponding inhibitors.

The highly sensitive determination of organophosphoric warfare agents is possible with a fluorimetric method established by Matousek [178] using horse blood serum-ChE and indoxylacetate. The excitation of fluorescence takes place with UV light of 355 nm; the maximum of the excited bluish green fluorescent light is obtained at 465 nm. Control tests and specimen are measured three times each at short intervals. From the comparison of the speeds ($V = \Delta\Phi/\Delta t$ (rise of the curve) for the control experiment (VK) and the specimen (Vp) the inhibition coefficient I is determined:

$$I = \frac{Vp}{VK}$$

On the basis of the value obtained I from previously established calibration curves it is possible to determine the concentration of the inhibitor. The limiting concentration indicated for sarin and soman are 1 by 10-7 mg/ml, for VX 2 by 10-6 mg/ml.

29.2.3.1. Detection and Semiquantitative Determination of Organophosphoric Inhibitors in Water with the Substrate-Indicator Paper [136]

Production of the Substrate Indicator Papers:

1.70 gr butyrylcholineiodide and 0.30 g bromothymol blue indicator are resolved in 100 ml 96 percent ethanol. This solution is used to impregnate chromatography paper (surface weight 100 g/m2) by dipping it for 30 s, allowing it to drip and to dry in the horizontal position in the air. After drying it is cut into rectangular pieces of exactly 15 mm by 20 mm by removing the border strips about 5 mm wide and these rectangular pieces will be used for the determinations. 100 ml of the solution are sufficient to produce about 1500 papers. The papers can be kept indefinitely. For the determination we must use each time only papers from the same production batch.

Reagents:

Enzyme solution

A 2 percent solution of lyophilized horse blood serum in Michel buffer, pH 8.0 (0.742 g sodium veronal, 0.082 g potassiumdihydrogenphohsphate and 17.535 g sodium chloride are dissolved in 1 l water free from carbon dioxide, with 0.1 normal hydrochloric acid and the pH value of the solution is adjusted to 8.0). When not in use it is stored in the refrigerator.

Reference Buffer pH 7.0

A phosphate buffer according to Sorensen is used. The production takes place by mixing 39 ml of a solution of 0.078 g potassium hydrogen phosphate in 1000 ml water and 61 ml of a solution of 11.876 g disodium hydrogen phosphate in 1000 ml water.

Procedure:

First the suitability of the paper produced is tested. 0.05 ml each of water and enzyme solution is carried by pipett to a point of a microobject carrier, then 0.1 ml reference buffer is added with the pipett. On both points a substrate indicator paper is placed. The second object carrier is used to cover and the time taken for both papers to have the same color is noted. It should be between 3 and 7 minutes. If it is not in this range, the acid content of the papers or the indicator may be the cause. In this case fresh papers must be produced using a different quality of paper or after adding 0.1 normal sodium hydroxide to the substrate indicator solution until the latter becomes olive brown.

Preparation of the Specimen Water:

The specimen water must be neutralized before the detection. To this end 10 ml are treated with 5 drops of a solution of 0.1 percent bromothymol blue in 20 percent ethanol and adjusted by careful addition of 0.01 normal sodium hydroxide or hydrochloric acid to a greenish-blue shade.

For specimen waters of high degree of hardness differences can arise in the change of color as compared with the control specimen through the buffer effect of the salts contained in the water giving rise to wrong results. Therefore it is proper to use instead of the distilled water for the reference value a part of the specimen water decontaminated with active carbon. The decontamination takes place by adding a generous amount of powdered active carbon with the spatula tip to 5 ml of the specimen water; it is agitated several times within a few minutes, then filtered and neutralized. With this treatment the poison concentration drops by about 3 powers of 10.

Execution of the Detection Process

Two drops of 0.05 ml enzyme solution each are placed with a pipett on a microobject carrier. The left-hand drop is treated with 0.05 ml sampling water, the right-hand one with 0.05 ml reference water. Agitation is carried out with 2 thin glass rods which must be separated strictly from each other and always used on the same side. After an incubation time of 5 minutes for sarin, soman, tabun and DFP or 15 minutes for VX both sides are covered with a paper each and a second object carrier is applied. Whereas the paper on the right-hand side (comparison) gradually changes its color from blue-green to green, yellowish-green, lemon yellow to bright yellow, the left paper (specimen) in the presence of organophosphoric inhibitors remain bluish-green or changes its color much more slowly.

Execution of the Semiquantitative Determination:

The procedure is similar to that of detection only in addition between the specimens and the reference at the center of the object carrier 0.1 ml reference buffer is introduced with a pipette and also covered with a paper after the incubation period. The time elapsing from the application of the paper (the specimen and reference are covered simultaneously with paper, then the buffer) until the color of the reference and the specimen with the buffer is obtained. From this we calculate the inhibition by the following formula:

$$100 - \frac{to \cdot 100}{tp} = \text{percent inhibition;}$$

to = working time of the reference
tp = working time of the specimen

For the individual inhibitors calibration curves are established, while the percentage of inhibition obtained for certain concentration is plotted against the negative logarithm of the concentration. In the central area of the curve (20 to 80 percent inhibition) with exact procedure a very high precision is achieved. An advantage of this method of detection and determination is that the temperature hardly influences the values, the duration of the determination changes mainly, because of the dependence of the speed of the splitting of the substrate on the temperature. The sensitivity of the method is 1 by 10-6 mg/ml for sarin and soman, and 5 by 10-6 mg/ml for tabun.

The central area of the calibration curves from 20 to 80 percent inhibition includes a concentration range of the organophosphoric inhibitors of about 1 power of 10. In many cases it is therefore necessary to start a dilution series. If therefore the paper on the specimen side does not change in color or the working time is more than 30 to 40 minutes, the specimen water is diluted each time 1 to 10 with water free from poison

and an experiment carried out with each dilution stage. The following example should illustrate the calculations needed to evaluate such a determination.

The sarin concentration in a sample water is to be determined. In the experiment carried out with the undiluted sample water the paper did not change its color. The sample water is therefore diluted twice, each time 1 to 10. With the first dilution no measureable working time could be obtained, on the other hand the second dilution gave a working time of 10 minutes. The working time of the reference specimen is obtained as 4 minutes.

The inhibition is calculated from:

$$100 - \frac{4 \cdot 100}{10} = 60 \text{ percent inhibition.}$$

From the calibration curve for sarin for this inhibition a value pI (= negative logarithm of the sarin concentration in milligrams per milliliter) of 5.2 was noticed. The conversion is then carried out as follows:

Sarin concentration = 10-pI
= 10-5.2

lg sarin concentration = 0.8-6
sarin concentration = 6.3 by 10-6 mg/ml

Since the sample water was diluted altogether 1 to 100 it was found that it contains:

6.3 by 10-6 by 100 = 7.3 by 10-4 mg/ml

or
0.63 mg sarin/l.

29.2.3.2. Photometric Determination with Indophenylacetate as Substrate /160/

Reagents:

A. Enzyme buffer solution

Solution of lyophilized horse blood serum in 0.05 molar phosphate buffer of pH 8.0 (40 cholineesterase units according to Ammon per milliliter).

B. Substrate solution

0.08 g indophenylacetate[8] (melting point 115 to 116 degrees C from petroleum ether /124/ are dissolved in 100 ml methanol (3.3 by 10-3 molar solution).

C. Phosphate buffer pH 8.0 according to Clark and Lubs

46.8 ml 0.1 normal sodium hydroxide and 50 ml 0.1 normal potassium dihydrogen phosphate solution are mixed and water is added up to 100 ml.

[8] N-(4'-acetoxyphenyl-)p-quinonimin.

Procedure:

1 ml of the aqueous poison solution is mixed in a test tube with ground stopper with 4 ml enzyme buffer solution (A) and incubated in the thermostat at 30 degrees C in the case of sarin, soman, tabun, TEPP or 30 minutes for the V warfare agents or insecticide phosphoric acid esters. After this period 0.15 ml substrate solution (B) are added. The tubes are left another 30 minutes in the thermostat, then the enzymatic reaction is either timed by adding 1 drop of DFP solution (5 mg/ml) and the measurement carried out subsequently or the extinction is measured immediately after the 30 minutes.period. The measurement takes place in the spectralphotometer at 625 nm against a reference specimen which was set up with 1 ml water and 4 ml phosphate buffer (C) parallelly to the specimen. The determination of the inhibitor concentraction in the specimen takes place on the basis of a calibration curve which was recorded with pure substance and in which the inhibitor concentration was plotted against the extinction. The erythrocyte esterase (AChE) more suitable for the determination of some organophosphoric inhibitors can be used in this method; the splitting of the substrate takes place at approximately the same speed.

Instead of the indophenylacetate 2,6-dichlorindophenylacetate can be used; the measurement of the extinction takes place in this case at 610 nm.

29.3. Biochemical Determination of Organophosphoric Inhibitors Using Other Enzymes

The aliesterases contained in the liver of mammals may be used in many ways primarily for the enzymatic detection of organophosphoric inhibitors on paper and thin layer chromatograms. We are dealing with hydrolases which are able to split particularly the esters of short chain aliphatic fatty acids. The substrate used mainly are α- and β-naphthylacetate; the free naphthol obtained in the enzymatic splitting is associated with a stable diazonium salt, for example the fast blue salt B, in a dye.

The inhibition of steapsinlipase through sarin and systox allows the fluorometric determination of these inhibitors using dibutyrylflorescein as substrate [162]. The work is carried out with the 5 by 10-5 molar concentration of the substrate for a pH value of 8.0; for an incubation time of 2 minutes it was possible to determine 3 by 10-5 to 3 by 10-4 mg of the inhibitors per milliliter.

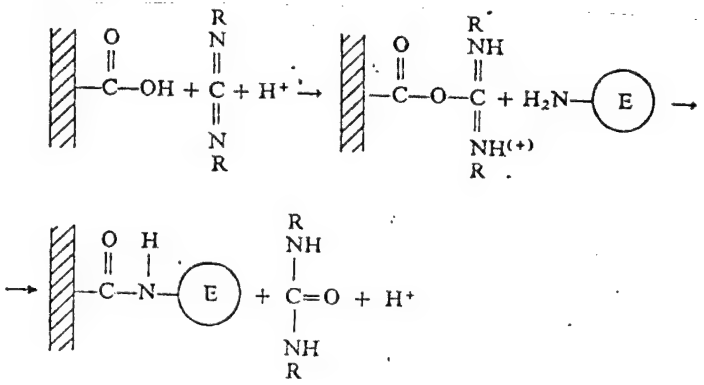
Some ferments in which the substrate splitting is accompanied by the evolution of gas are inhibited in their activity by the organophosphoric inhibitors. The reduction in the amount of gas evolved in the unity of time and whose measurement in the fermentation tube can be achieved with higher sensitivity by adding foam producers is a measure of the inhibitor concentration. Suitable ferments are catalase with hydrogen peroxide as substrate, urease with urea and carboxylase with brenzoracemic acid as substrate [163].

29.4. Carrier Fixed Enzyme and Enzyme Electrodes

The low stability and the relatively high cost combine with the fact that a given amount of enzyme has only been used once are factors which limit the wide use of the enzymes even for analytical purposes. Although the enzyme if no inhibitor were present is fully active once again after completing the reaction as catalyst, up to now it was satisfied each time. These drawbacks can be avoided to a great extent if the enzymes

are bound in a high molecular and generally insoluble carrier. The action center of the enzymes is not concerned in the bond and thus it occurs free so that the substrates are bound and converted more or less without obstacles and the reaction products can be released. By this manner it is possible to stabilize the enzymes and use them more extensively than before because of the possibility of repeated use as catalysts. In particular the thermal stability is increased by inhibiting the autolysis (=self-dissolution). Another advantage is that the reaction sediment remains free from photenes when carrier fixed enzymes are used.

The most important method for carrier fixing of enzymes is the covalent bound on lateral or synthetic carrier substances through reactive groups of the protein, while a large number of possibilities exist. This principle will be elucidated on a typical procedure- In the binding on carboxyl groups of the carrier by means of carbodiimides, the following reactions take place.



Further possibilities of carrier fixing are the adsorption of the enzymes on inert carriers (cellulose, glass, silica gel, organic polymers among others), the electrostatic binding on ion exchangers or copolymerization. For the permanent use of carrier fixed enzymes in automated warning and monitoring plants special requirements are imposed on the preparations. The enzymes must be firmly bound; thus they must not "bleed". The carrier should absorb neither the substrates and inhibitors nor their decomposition products; it should not affect the pH value and must not be attacked by microorganisms.

29.4.1. Carrier Fixed Cholineesterases and their Analytical Application

The first experiments for the application of carrier fixed cholineesterase for the detection of organophosphoric inhibitors were carried out by Bauman and his colleagues [154]. At the same time interesting possibilities were shown for the development of biochemical detectors for organophosphoric chemical warfare agents. The immobilization of ChE (from horse blood serum) is caused by the dissolution in a starch solution, impregnation of a polyurethane foam pad with this solution and subsequent freeze drying. The enzyme remains stable on the pad for 12 hours and showed no extensive decrease of activity. The enzyme pads cut in two a smaller format were used in connection with the electrochemical system described in section 29.2.2. If the butyrylthiocholineiodide solution flows through the pad located between two platinum electrodes, the electrochemical system shows a low potential when a current is applied because of the oxidation of the thiocholine obtained by enzymatic hydrolysis into disulfide on the anode. If the substrate

solution or in air current sucked up simultaneously by the pad containing an inhibitor, the activity of ChE decreases and the potential increases until in case of total inhibition of the enzyme the potential reaches the oxidation of the iodine ion.

These enzyme pads represent together with -naphthylacetate as substrate in a suitable arrangement of the apparatus a possibility for continuous control of air and water for the presence of organophosphoric inhibitors [156]. The constant fluorescence measured with a uniform substrate flow through -naphthol is reduced or extinguished in the presence of inhibitor in the sample water additionally mixed constantly in the substrate solution. A carrier fixed ChE preparation more stable for use was obtained by incorporation in polyacrylamide gel [179]. The control of the activity using N-methylindoxylacetate in tris-buffer (pH 7.4) shows that the preparation was stable with dry storage over 80 days and with discontinuous use for 40 hours.

By covalent coupling of AChE of silanized fritted glass discs very stabile preparations were obtained [180]. The silanization of glass was accomplished with γ-aminopropyltriethoxysilane. By means of the carbodiimide methods then the AChE was coupled with a covalent bond between the amino groups of the aminoalkylsilane glass obtained and the carboxyl group. The enzyme retained in this form in activity over a period of 55 days, while between the different applications the discs were kept each time in phosphate buffer. It was possible to reactivate the original activity to 70 percent for a preparation totally inhibited with paraoxon by treatment with toxogonin.

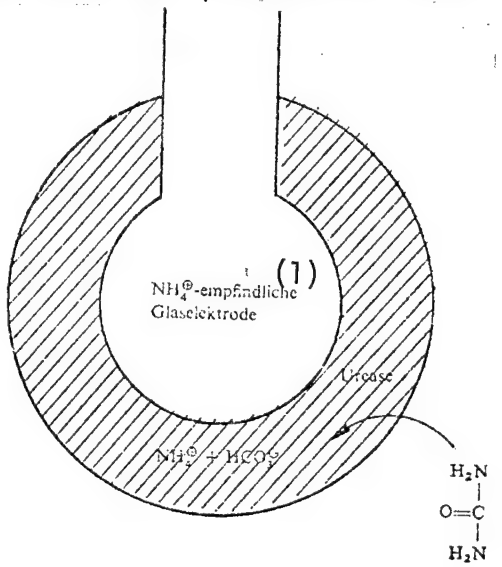


Figure 29.3. Enzyme electrode to determine urea according to the method of Guilbault [181]. Key: (1) NH4 sensitive glass electrode

29.4.2. Enzyme Electrodes

One of the forms of application of carrier fixed enzymes is the use of enzyme electrodes. The latter are based on the fact that the product of the enzymatic substrate separation is often a simple ion, to which the conventional ion selective electrodes react. Since the enzyme effect is highly elective, these enzyme electrodes make it possible to determine individual components in complicated mixtures without previous separation.

One prototype for these electrodes is the enzyme electrode for urea described by Guilbault [18]. The enzyme urease is fixed in a layer of polyacrylamide gel which is held by a nylon fabric or a cellophane membrane on a glass electrode selective for ammonium ions. The urea diffuses in the gel layer; the ammonium ions formed by enzymatic splitting are indicated by the electrode.

So far no report has been made on enzyme electrodes for the detection of poisons, but the development of such electrodes for example for organophosphoryls on the basis of phosphatases combined with the phosphate selective electrodes described already in the literature is quite imaginable.

Check Questions

1. What are the advantages and drawbacks of the biochemical methods for the detection and determination of chemical warfare agents?

2. Describe the known types of esterases separating cholinesters on the basis of their important distinguishing features.

3. Explain the differences in the inhibiting effect of the different organophosphoryls on the cholineesterases.

4. What is the importance of the determination of the activity of cholineesterase in human blood serum?

5. Define the values I50 and pI50.

6. What are the possibilities for determining the I50 value of an inhibitor?

7. Define the "cholineesterase unit" for the cholineesterase activity obtained according to the manometric, electrometric and calorimetric methods.

8. Explain why in the determination of inhibitors the enzyme must first be incubated with the inhibitor and then the substrate added.

9. Why is it necessary to treat parathion with bromine water before the biochemical determination?

10. What is the basis of the determination of the organophosphoric warfare agents with the substrate indicator paper, and how is it to be carried out?

11. What are the advantages of the use of carrier fixed enzymes?

12. Name the possibilities of application of carrier fixed enzymes in the analysis of organophosphoric warfare agents.

30. Biological Methods

30.1. Determination of LD and LC Values

To characterize and compare the toxicity of poisons the data on defined doses or concentrations are used preferably. These data allow very exact and objective comparisons when the investigation were carried out with the same type of application, the same type of animal (while the species of animals, sex, age and weight of the group of animals had to be uniform) and same experimental conditions (time of effect of the poison, environmental conditions, feed, etc.).

The doses used most frequently are the average lethal dose (LD 50), the lethal dose (LD 100) while the data on LD 99 are more exact for statistical reasons as well as the average effective dose (ED 50). Similarly the average lethal concentration (LC 50) and the lethal concentration (LC 100 or LC 99) are indicated.

The LD 50 is defined as a dose which leads to death under defined conditions for 50 percent of the animals used, the LD 100 causes death in all the animals used. The ED 50 gives the dose at which a certain definite effect or reaction can be recorded in 50 percent of the test animals used. The data are given generally in milligrams or grams substance per killogram body weight (mg/kg). All types of application are used.

To determine experimentally the doses or concentrations groups of at least 8 to 10 test animals are administered under constant comparable conditions definite doses of the substance to be studied. The number of animals dying in each group is recorded after corresponding times and statistical or/and graphic methods are used to derive the LD 50 (or other doses and concentrations).

Besides the purely statistically methods [185] or graphic methods of calculation [297] it was decided to determine the doses indicated with a combined graphic and statistical method [298]. To this end the dead animals per group are indicated in percentage of the total number of the animals used and represented in a probability system while the ordinates represent the percentages and the abscissa the dose values in logarithmic scale. The measurement points are joined with each other with a straight line. After correction of the latter by means of a tabulated value it is possible to obtain on this basis the desired dose as well as by additional calculations the limits of reliability for a pregiven probability of error and assure their statistical certainty. The method offers as compared with others the advantage that for a sufficient number of test animals per group relatively exact, statistically certain results can be obtained.

To describe the effect of the antidotes or other measures for protection against poisonings the rate of involution of the LD 50 is often indicated. We mean by this term the quotient of the LD 50 obtained by treatment or prophylaxis and the LD 50 without therapeutic or prophylactic measures. Both doses must be obtained under comparable conditions. The extent of the involution rate is a measure of the efficacy of the protective measures against poisoning.

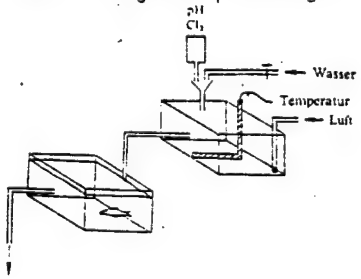


Figure 30.1: Device for water control with fish [182]

30.2. Use of Animals for the Detection of Warfare Agents

Similarly to the use of the subjective powers of observation of man for certain warfare agents an attempt was made especially during the period of the First World War to use the higher sensitivity of the organs of sense of some animals or their physiological reactions to detect the warfare agents. The highly sensitive sense of smell of the dog is for example able to detect even concentrations between 0.1 µg/l of sulfur yperite vapors. It was also possible to train the smelling capacity of dogs and their reaction to differentiated indication of warfare agents as compared with other smells [181]. Cats showed an increased toxic sensitivity to phosgene. The chicken reacts with even greater sensitivity to the slightest traces of phosgene, as it may generally be established that birds show with maximum sensitivity physiological reactions in the presence of poisons in the atmosphere. Canaries can therefore be used as living indicators of prussic acid and carbon monixide. Similarly the American Army during the First World War used snails which in the presence of low concentrations of yperite in the air gave a milky secretion.

Although the use of animals for this purpose has many drawbacks which are due to the lack of the possibility of understanding or the difficulty of evaluation of the behavior of animals deviating from the normal, this possibility is also being studied at present. As is apparent from the civil toxicological and foreign literature on military chemistry, this applies particularly to the identification of the organophosphoric warfare agents and insecticides in water and foodstuffs. Dogs are trained to signal concentrations more than 0.5 mg/m3 of soman, sarin, VX, tabun and CS [300].

Besides the use of mice to obtain toxic data on the compounds, which was described in greater detail in section 30.1, these animals are also used to establish poisons in foodstuffs by means of experiments with feed or by injection of extract. Some species of ornamental fish who show an extremely high toxic sensitivity for organophosphoric warfare agents and insecticides are used to detect these poisons in drinking water and with a suitable device (Figure 30.1) for constant water control in larger processing plants [182, 183].

The LC 50 values for sarin may be found for different periods of effect in Table 30.1.

Since the fish especially in the case of water of low hardness react sensitively to chlorine, the latter is reduced by adding suitable concentrations of sodium thiosulfate (7 mg Na2S2O3 per 1 m chlorine). The temperature is kept at 20 to 28 degrees C, the oxygen content at more than 4 mg/l and pH value regulated between 5 and 9. The particularly suitable types are the Pimephales promelas which give very characteristic reactions at low concentrations of the poison (extension of the fins forward, spreading of the gill cover). A concentration of 0.5 mg sarin/l causes in these fish after 6 minutes 50 percent loss of balance in 50 percent of them and after 12 minutes death in 50 percent. In the Leponis macrochirus with a longer period of effect (30 days) it is still possible to detect 0.1 µg parathion/l. Guppies are suitable also to detect traces (5 mg/l) of sulfur yperite dissolved in water [184]. During the Vietnam War the inhabitants of jungle villages after the suspected use of chemical warfare agents and poisons had waited for the reaction of the fish to judge the potability of drinking water.

Table 30.1

Species of Fish	Water	LC 50 in g/l 24 h	48 h	96 h
Pimephales promelas	soft	6.5	5.3	4.4
	hard	32.1	31.9	31.9
Lepomis cyanellus	soft	4.6	4.2	4.2
	hard	15.2	15.2	15.2
Guppies (lebistes reticulatus)	soft	8.3	7.2	7.2
	hard	21.0	14.5	13.8
Lepomis macrochirus	soft	7.5	3.2	3.2
	hard	23.5	23.5	23.5
Carassus auratus	soft	16.1	11.8	9.8

To determine the residue and the insecticides containing phosphorus in plants and foodstuffs it is possible to use fly, leeches and water fleas. The flies are placed mostly in contact with an extract in flat covered glass dishes, the leeches are placed in an aqueous extract. The achievable sensitivities are very high. Since the values depend on the conditions, an indicator value is for example that for a period of effect of 24 hours it is possible that with the leech to detect still 5 µg parathion (after prior oxidation) in 50 ml aqueous solution through the death of the animals. It is interesting to detect toxic organic phosphorus compounds on paper chromatograms by contact of mosquito larvae with the chromatogram divided into regular sections.

Check Question

1. In what form can animals be taken to detect chemical warfare agents and other poisons in water?

31. Physical and Physical-Chemical Methods

The determination of physical constants is used in the analysis of unknown specimens for identification, in the laboratory investigation of known substances it is carried out to control the purity and for synthetic products also to confirm the identity. For solid substances the melting point, in liquids the boiling point, the specific weight, the vapor pressure and the index of refraction are determined. In each case the substance is purified extensively before determining the constants by recrystallization, sublimation, distillation or by preparative chromatography.

The spectral analytical study, especially in the infrared region of the spectrum offers the most perfect possibility for describing known and identifying unknown substances. The infrared spectrum of a pure substance is quite comparable in its specificity with the evaluation of a fingerprint in criminal investigations.

Although the physical methods are inferior to the chemical ones of analysis as regards precision, they offer significant advantages as regards shortening the time needed, reducing manual work and in the sensitivity. Table 31.1 which was taken from the publication by H. Kienitz /232/ allows the comparison between some chemical and physical methods of quantitative analysis.

31.1. Determination of the Specific Weight

For the determination of specific weight of liquid warfare agents the pycnometer is suitable. The following weights are determined:

a) pycnometer, filled with substance = A
b) pycnometer, filled with water = B
c) empty pycnometer = C

From this we calculate the density in g . cm-3

$$\varrho = \frac{A - C}{B - C}$$

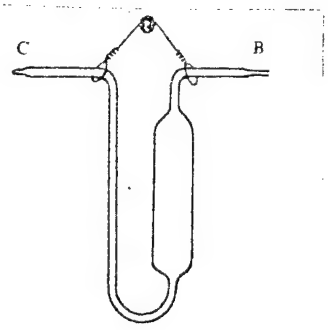


Figure 31.1: Pycnometer according to Sprengel/Ostwald.

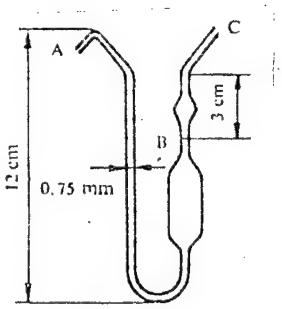


Figure 31.2: Self-filling pycnometer according to Hennion /225/

Table 31.1. Comparison Between Some Quantitative Methods of Analysis

Methode (1)	analytische Aufgabe Nachweis oder Bestimmung (2)	Probenmenge (3)	ungefähre Erfassungsgrenze (4)	Wirkungsgrad der Methode (5)	Genauigkeit des Analyseergebnisses (6)	Dauer der Analyse (ohne Vorbereitung) (7)		
						manuelle Arbeit (8)	Auswertung (9)	Bestimmungsdauer einschl. Auswertung (10)
		ohne Anreicherungsverfahren (11)						
		P in mg	E in mg	W = P/E	rel. in %	in min	in min	in min
anorganische Makrogewichtsanalyse (12)	Elemente (22) Anionen (23) Kationen (24)	100 ... 500	0,1	10^3	0,2 ... 0,5	20 ... 120	2 ... 5	60 ... 300
anorganische Mikrogewichtsanalyse (12a)	dto.	3 ... 10	0,002	10^3 ... 10^4	0,5	30 ... 120	2 ... 5	60 ... 300
anorganische Makromaßanalyse (12)	dto.	20 ... 500	0,1	10^3 ... 10^4	0,2 ... 0,5	2 ... 20	2 ... 5	5 ... 25
anorganische Mikromaßanalyse (12)	dto.	3 ... 10	0,001	10^3 ... 10^4	0,2 ... 0,5	2 ... 20	2 ... 5	5 ... 25
Kolorimetrie (13)	dto.	0,1 ... 1	0,0001	10^3 ... 10^4	1 ... 2	2 ... 5	2 ... 5	5 ... 10
organisch-chemische Makroanalyse (14)	funktionelle Gruppen, Moleküle (25)	200 ... 1000	0,1	$2 \cdot 10^3$ ... 10^4	0,2 ... 0,5	10 ... 240	5 ... 10	15 ... 250
organisch-chemische Mikroanalyse allgemein	dto.	3 ... 10	0,002	10^3 ... 10^4	0,2 ... 0,5	5 ... 150	5 ... 10	10 ... 160
organisch-chemische Mikroelementaranalyse (14a)	C, H, N, O, S, Cl anorganische Verbindungen (26)	3 ... 5	0,01	10^{+3}	0,3 ... 1	20 ... 40	2 ... 5	25 ... 45
anorganische und organische Tüpfelanalyse (14b) (15)	Elemente, Anionen, Kationen, funktionelle Gruppen (27)	0,01 ... 0,1	0,00001	10^3 ... 10^4	qualitativ (30)	1 ... 2	–	1 ... 2
Gaschromatografie (16)	Moleküle (28)	0,5 ... 10	0,0001	10^3 ... 10^5	1 ... 3	5 ... 60	5 ... 15	10 ... 75
IR-Spektroskopie (16a)	funktionelle Gruppen (29)	0,5 ... 5	0,005	10^2 ... 10^3	1 ... 2	5 ... 10	10 ... 30	15 ... 45
Flammenfotometrie (17)	Elemente	1 ... 10	0,0005	10^3 ... 10^4	1 ... 2	1 ... 2	2 ... 5	3 ... 10
Emissionsspektrometrie (18)	Elemente (22)	0,1 ... 1	0,00005	10^4 ... 10^5	2 ... 3	1 ... 2	2 ... 10	3 ... 15
Röntgenfluoreszenzspektrometrie (19)	Elemente	100	0,01	10^4	2 ... 5	1 ... 2	2 ... 10	3 ... 15
Massenspektrometrie (20)	Elemente, (22) Moleküle (28)	0,1 ... 10	0,0001	10^3 ... 10^5	1 ... 2	10 ... 20	10 ... 50	15 ... 60
Neutronenaktivierungsanalyse (21)	Elemente (22)	1 ... 100	10^{-8}	10^6 ... 10^8	5 ... 10	10 ... 60	15	25 ... 1000 Bestrahlungsdauer 10 ... 1000 min (31)

Key:
(1)method
(2)analytical problem detection or determination
(3)amount of specimen
(4)approximate detection limit
(5)degree of efficiency of method
(6)precision of the results of analysis
(7)duration of analysis (without preparation)
(8)manual work
(9)evaluation
(10)determination of duration including evaluation
(11)without enrichment
(12)inorganic macroweight analysis
(12a)inorganic microweight analysis
(13)colorimetry
(14)organic and chemical macroanalysis in general
(14a)organic and chemical microanalysis in general
(14b)organic and chemical microelementary analysis
(15)inorganic and organic spot analysis
(16)gas chromatography
(16a)IR spectroscopy
(17)flame photometry
(18)emission spectrometry
(19)x-ray fluorescence spectrometry
(20)mass spectrometry
(21)neutron activation analysis
(22)elements
(23)anions
(24)cations
(25)functional groups, molecules
(26)inorganic compounds
(27)elements, anions, cations functional grps
(28)molecules
(29)func. grps.
(30)qualitative
(31)time of irradiation

The ordinary pycnometers have the considerable drawback when working with warfare agents that some liquid mostly emerges when the capillary is placed on the slide. The pycnometers according to Sprengel/Ostwald are more suitable (Figure 31.1). The liquid is sucked up through the opening A (rubber ball or glass injection flask with rubber hose) in B there is a mark to which the liquid is adjusted by swabbing the excess liquid in A with a cell plug swab. It is very advantageous when working with toxic liquids to have the cell filling pycnometer according to Hennion [225] which can easily be made with a complete pipette of suitable size (Figure 31.2). The capillary tip A is dipped in the liquid; after the pycnometer is full to the calibrated mark B, the opening C is closed with a finger and after wiping the tip A it can be weighed.

31.2. Determination of the Melting Point

To determine the melting point the substance is filled in a glass melting point capillary and the latter is heated in the glass equipment according to Thiele in a sulfuric acid bath or for chemical warfare agents preferably in the copper or aluminum block. The filling in the capillary takes place over a glass plate which is subsequently decontaminated. It is very advantageous for rapid determination of the melting point in warfare agents which decompose with slower heating, to use a heating bench proposed by Kofler. It consists of a metal body about 4 cm wide and 30 cm long, at which a temperature gradient is produced by unilateral electrical heating. The temperature gradient is approximately linear. The substance is scattered directly over the chromium plated surface of the heating bench. According to the purity of the substance a more or less sharp limit is immediately clear between the liquid and solid phase. When working with melting point capillaries the determination of the melting point can be arranged exactly, by determining in a preliminary experiment the approximate position and in a second experiment adjusting the capillary only a few degrees below the value obtained in the equipment in the preliminary experiment. To determine the melting point in the presence of very small amounts of substance, a few crystals are needed, the work is carried out with a microheating table under the microscope with which it is also possible to identify much better the impurities in the specimen substance and the formation of eutectic mixtures with the main components than in the melting point capillaries. A substance is considered pure when the melting point after recrystallization has not changed by more than 1 degree C. A sure criterion of the identity of a substance is the mixed melting point. Equal parts of the specimen substance and the substance which could occur as a result of the melting point are mixed intimately by crushing. If the melting point of the mixture corresponds to the known substance of the mixture the specimen substance is identical to it.

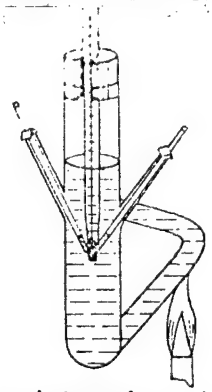


Figure 31.3: Glass equipment to determine the melting point according to Thiele.

31.3. Determination of the Boiling Point

The method to be chosen to determine the boiling point depends on the amount of the substance to be studied available. For field analysis warfare agent studies, the amount of specimen is generally very small and the use of a micromethod is necessary. A glass tube of about 6 mm diameter which is attached with a rubber ring on a thermometer is very suitable. The tube contains the capillary tube sealed at the upper end and dipping in the specimen liquid. When heating in the heating bath or with the burner when the boiling temperature of the specimen is reached a uniform current of fine vapor pearls is formed of the capillary.

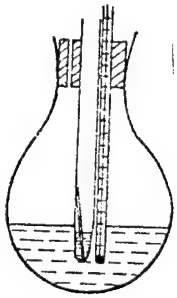


Figure 31.4: Determination of the boiling point.

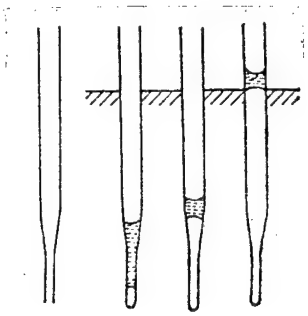


Figure 31.5: Boiling point tube according to Emich.

Another possibility is the determination with the boiling tube according to Emich. A capillary about 8 cm long of 1 mm internal width is drawn out with a microburner to a 2 cm long tip, with which it is possible to suck up some specimen substance.

Then the tip is melted in such a way that a small air bubble remains between the melted end and the liquid. The capillary is attached to a thermometer and introduced in a melting point equipment. When the boiling point is reached the air bubble rises to the liquid level of the heating bath. At that time the temperature is noted.

These methods described give reliable values only for pure substances which boil without destruction at normal pressure. For mixtures the boiling points of the components are determined during a fractionating distillation. The boiling point of chemical warfare agents which is very high under normal pressure (more than 200 degrees C) and which boil with destruction is obtained by vacuum distillation in a suitable microequipment. To evaluate the purity of the specimen it is sufficient to establish that under constant pressure most of it is transformed in a narrow temperature range. The exact determination of the boiling point of this substance under normal pressure is achieved by distillation at two different reduced pressures and calculation based on the dependence of the vapor pressure on the pressure.

The survey of the different methods of calculation and the constants needed for them may be found in the publication by Krell [202].

31.4. Determination of the Vapor Pressure

Two types of methods can be differentiated to determine the vapor pressure:

a) Static Methods

The substance to be studied is brought to a certain temperature which is to be kept constant and the vapor pressure adjusted with a thermal equilibrium of both phases is determined with a pressure measuring equipment.

b) Dynamic methods

The pressure is kept constant with a manostat and the temperature of the substance to be studied increased up to boil. The vapor pressure at boiling temperature is equal to the adjusted pressure.

The dynamic methods include the transfer method in which the gaseous phase corresponding to the measurement temperature which is in equilibrium with the liquid or solid phase of the substance to be studied, is recorded in amount. An indifferent gas current is sent through the substance to be studied. With suitably slow speed of flow the saturation pressure of the substance is adjusted as a partial pressure. The vapor carried is separated by condensation and freezing and can be determined by weighing or it is bound chemically and determined by a quantitative analytical method. The latter method is suitable for the determination of very low vapor pressures up to less than 10-4 Torr and was used also to determine the vapor pressure of sulfur yperite /226/. A simple method which is not very exact but is sufficient for many purposes is the determination of the boiling point at at least two different pressures. From a curve in which the log p (Torr) is plotted against 1/T (K-1), the vapor pressure can be interpolated for the different temperatures. To determine exactly very low vapor pressures methods have been described in the literature /204, 205/ which require a considerable apparatus cost. A simple device and convenient to operate described by Drewes allows the determination of the vapor pressure of liquids from 1 Torr at any temperatures. Only a small amount of mercury and the substance to be studied is needed and the measurement vessel can be produced so cheaply that it can be destroyed after use. The method can be applied in all laboratories which have an oil vacuum pump. Figure 31.6 shows the structure of the device. Figure 31.7 shows the measurement equipment. The measurement equipment is in a thermostatic bath during the measurement. The entrance opening of cock B is drawn into a fine nozzle. As shown in Figure 31.7 the measurement container is filled with mercury. After the vessel is connected with the instrument it is evacuated, and after the mercury level has dropped somewhat in the closed arm of the measurement equipment, it is aerated through the three-way cock C. By careful tilting the air bubble formed above the mercury is eliminated. Thereafter a few drops of the substance to be studied are added to the measurement vessel and sent over the mercury by tilting in the closed arm. After repeated evacuation and aeration the air bubble formed once again is removed; it does not matter that part of the substance here is also removed. Now the measurement instrument is tempered and the equipment once again evacuated. By means of the cock B the vacuum is regulated in such a way that the mercury in both arms is at equal level; the value read in the manometer D corresponds then to the vapor pressure of the substance at the temperature of the bath. By stagewise increase of the temperature and corresponding adjustment of the vacuum it is possible to determine the temperature dependence of the vapor pressure. If at very low vapor pressure the vacuum of the pump is not sufficient to bring the mercury level of the measurement level to the same height, we adjust to any exactly noted value of the scale on the measurement equipment and the vapor pressure is obtained from the difference of the millimeters read on the manometer d and on the scale of the measurement vessel.

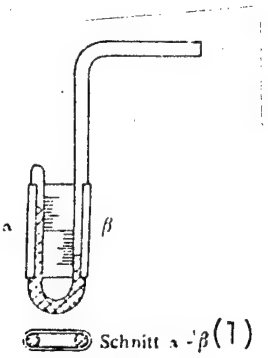


Figure 31.6: Equipment to determine vapor pressure according to Drewes.
Key: (1) pump

Figure 31.7: Measurement vessel to determine vapor pressure.
Key: (1) cross-section

31.5. Determination of the Index of Refraction

If the temperature and wavelength of the light are kept constant during the measurement of the index of refraction, the measured value can be considered within certain limits as a material constant and be used to characterize organic substances. The index of refraction can moreover be used to determine quantitatively the concentration of a solution or to analyze binary liquid mixtures.

By the combination of the index of refraction with another quantity dependent on temperature, the density, it was possible for Lorentz and Lorenz to introduce theoretically and practically with the so-called molar refraction an absolute constant dependent on the temperature only to a very small extent.

$$R_M = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{M}{\varrho};$$

n: index of refraction
M: defined or assumed molecular weight
ϱ: density

The molar refraction should be obtained according to their original theoretical concept by addition from the atomic refractions. Many practical studies have shown however that this additivity is only valid with good approximation since the effects which are more or less effective of the binding conditions and the molecular structure were not taken into account in their calculations. It was therefore recently decided to add the binding refractions. A survey of such binding refractions calculated mainly by Vogel /200/ and Sayre /201/ is found in Table 31.2.

Eisenlohr and Woehlisch shows that for the so-called molecular refraction coefficients M by n20D, therefore for the product of the molecular weight and the index of refraction measured at 20 degrees C for the line D, the same additive properties apply as for molar refraction. On this basis the constants indicated in column 3 of Table 31.2 were calculated from which, according to:

$$n_D^{20} = \frac{S}{M}$$

(in which S is the sum of the binding refraction constants) the index of refraction can be obtained.

Bindung (1)	Bindungs-refraktion Linie D, und 20°C (2)	Bindungs-refraktion Konstante (3)
P—C	3,575	25,57
P—Cl	8,856	68,57
P—H	4,010	16,16
P—O	3,102	28,11
P→O	−1,032	22,17
P—S	7,583	47,07
P→S	6,866	54,26
P—F		34,98
P—N		29,28
C—H	1,676	3,87
C—C	1,296	12,86
C=C	4,17	9,39
C—C (Sechsring) (4)	1,27	
C—C (Aromat) (5)	2,688	
C—F	1,55	
C—Cl	6,51	56,80
C—Br	9,39	124,51
C—O (Äther) (6)	1,54	
C—O (Azetale) (7)	1,46	
C=O	3,32	29,39
C=O (Methylketone) (8)	3,49	
C—S	4,61	32,84
C=S	11,91	
C—N	1,57	
C=N	3,76	
O—H (Alkohole) (9)	1,66	13,15
O—H (Säuren) (10)	1,80	10,54
S—H	4,80	
S—S	8,11	
S—O	4,94	37,1
S→O	−0,20	20,84
N—H	1,76	7,26
N—O	2,43	
N→O	1,78	
N=O	4,0	32,26
N—N	1,99	
N=N	4,12	

Table 31.2: Binding refractions and constants.
Key: (1) Bond; (2) Binding refraction, line D and 20 degrees C; (3) Binding refraction constant; (4) six-membered ring; (5) aromatic substance; (6) ether; (7) acetals; (8) methylketones; (9) alcohol; (10) acids.

The determination of the index of refraction is carried out with the Abbe refractometer. The instrument is here under the outlet. The cleaning is carried out with careful and thorough washing of the prisms with cotton plugs, which were impregnated with alcohol. The plugs are placed in a vessel and subsequently burned. The microrefractometer described by Jelley is very suitable, since work can be carried out with very small amounts of liquid. Its structure is very robust and it allows the determination from n = 1.33 to n = 1.92 with a precision up to the third decimals.

31.6. Methods of Spectral Analysis

The absorption spectroscopy in the visible and ultraviolet region of the spectrum is one of the oldest physical methods to clarify the structure of organic compounds and in the form of visual colorimetry and photometry one of the most commonly used methods to determine the concentration of colored or fluorescent compounds. The absorption of light in this spectral range, that is from 400 to 1000 nm (visible region) and from 200 to 400 nm (UV) is caused by the excitation of the loosely bound valency electrons into excitations. Such molecules show an absorption which contain chromophoric groups[9], through conjugation of several of these groups and simultaneous presence of auxochromic groups[10] it is considerably reinforced. The firmly bound valency electrons in the region below 200 nm, the so-called vacuum or Schumann UV is excited into oscillations and excitations of the still firmly bound inner electrons close to the nucleus take place only in the x-ray range.

The spectra in the visible and UV range are not very marked and have mostly only a few maxima and minima. Limits are therefore imposed on their use to characterize organic compounds. An important advance was obtained by the opening of the infrared or ultrared region of the spectrum or molecular spectroscopy which was rendered possible by the improvement of the thermal elements acting as detectors for thermal radiation to the maximum sensitivity and the extensions of the resolving capacity of the monochromators.

The infrared region extends from the red end of the visible region to the region of short electromagnetic waves. The region from 0.75 to 3 by 10-6 is called near infrared, from 3 to 40 by 10-6 m as middle infrareds, then follows the remote infrared. The absorption in the near and middle range is based on the consistency of the oscillation frequencies of the molecules with the frequencies of the waves of this region. In the remote infrared the rotation movements of the molecules around the main axis of inertia are effective. In the same spectral region the Raman spectra are also measured. Their occurrence is based on the polarization of the molecules through oscillations and rotation. The Raman spectra are found in the spectrum of scattered light besides the frequencies of the primary light. They are just as important for investigating the structure of the molecules as the infrared spectra, but their measurement requires considerable cost for equipment because of the low intensity and has not yet been

[9] Because of the presence of chromophoric groups in the molecule a compound appears colored. Examples of such groups are the nitro group (-NO2), the azo group (-N=N-) or systems with conjugated double bonds, that is double bonds within which there is a simple bond.

[10] The auxochromic groups designate in the dyestuff theory substitutes with free electron pairs which by their entrance into the chromogens (aromatic radicals, which are colored with a chromophoric group) cause a reinforcement of the color intensity and a deepening of the color. Examples are the amino group (-NH2) and the dimethylamino group (-N(CH2)2).

generally introduced. As compared with the spectra in the visible and ultraviolet range the infrared spectra, even for substances for simple structure, in accordance with the many theoretically calculated possibilities for oscillation and rotation are very rich in maxima and minima and their evaluation requires a lot of experience.

The absorption spectroscopy can be used for the identification of unknown and the determination of known substances, in this last case the intensity depending on the concentration of a maximum characteristic for the substance to be determined is measured.

The photography of the UV spectra takes place in UV-permeable dishes in dilute solution in a nonabsorbing solvent, for example n-hexane. IR spectra of gaseous and liquid substances are recorded using dishes, whose windows consist of the halides of alkaline and earth alkali elements. Solid substances are mixed with potassium bromide and compressed and the blanks are introduced in the IR spectrographs.

Besides the high specificity the methods of absorption spectral analysis have the further advantage that the specimen substances are not changed by the measurement and are available for further studies. The recording of a spectrogram for the purpose of identification and characterization of a compound is naturally only practical if it is possible to obtain the specimen substance with highest purity.

31.6.1. UV Spectroscopy

Extensive studies on the characterization and determination of chemical warfare agents by UV spectroscopy were carried out from 1936 to 1944 by Mohler [287]. Different authors had established the UV spectroscopy for determining the organophosphoryls, including parathion and sarin [288] and other poisons. A survey of some of the characteristic absorption maxima in the UV and the vacuum UV given in the literature for some warfare agents and poisons may be found in the following table:

Compound	λ in nm	Compound	λ in nm
bromacetone	209	ethyldichloroarsine	249
bromomethylethylketone	212	2-chlorovinyldichloroarsine	214
ω-chloracetophenone	198	bis-(2-chloroethyl)-thioether	202
xylylbromide	199	parathion	274
bromobenzylcyanide	about 244	paraoxon	270
diphenylchlorarsine	271	DFP	261
diphenylcyanoarsine	226	LSD	312
adamsite	198	N-methyl-3-piperidylbenzilate	258
chloropicrin	205		

Table 31.3: Absorption Maxima in UV

Table 31.4: IR Absorption Bands for Organic Phosphorus Compounds

Frequenz (cm^{-1}) (1)	Struktur (2)	Schwingung (3)	Art (4)
460 ... 574	P—S—X (X = H, C, P)		
493 ... 617	P—S—X (X = H, C, P)	P—S	
420 ... 587		P—Cl	
513 ... 565	O=P—Cl	P—Cl	
(5) etwa 515	S—P—S—C	P—S	
526 ... 649	P—S—H	P—S	V
etwa 538	S—P—S—C	P—S	V
570 ... 600	$R_1R_2R_3C$—S	C—S	
etwa 571	O—P—S—C	P—S	V
600 ... 700		C—S	
600 ... 750	P=S	P=S	
600 ... 630	R_1R_2CH—S		
etwa 606	O—P—S—C	P—S	V
625 ... 645	C—SP	C—S	V
630 ... 660	R—CH_2—S		
650 ... 750	P-Alkyl	P—C	(11) V
685 ... 705	CH_3—S		
702 ... 730	P—NR_2	P—N	V
etwa 715		P—N	
720 ... 750	P—O-Alkyl	P—O	sym. V
740 ... 800	P—F	P—F	(7) 3-wert. P
787 ... 840	P=S	P=S	V
788 ... 820	P—O-Alkyl	P—O	V
815 ... 940	P—F	P—F	(8) 5-wert. P
etwa 800	$(CH_3)_2C$		(9) Gerüstschw.
865 ... 940	O=P—F		
909 ... 971	P—O—C_3H_7—i		
925 ... 1005	Zyklohexan (6)		(10) Ringschw.
900 ... 980	P—O—P	P—O	
950 ... 1018	P—O—CH		
970 ... 1025	P—O-Alkyl	P—O	(14) asym. V
987 ... 1042	P—O—CH_2		
989 ... 1006	P—N—C	N—C	V
995 ... 1050	P—O-Alkyl	O—C	V
1000 ... 1055	Zyklohexan		Ringschw.
1015 ... 1060	P—O—CH_3		
etwa 1030	P—O-Aryl		
1040 ... 1060	—S=O		
1087 ... 1190	CH—O—P		
1105 ... 1170	CH_2—O—P		
1109 ... 1114	P—O—C_3H_7—i		

Key: (1) frequency (cm-1); (2) structure; (3) oscillation; (4) type; (5) about; (6) cyclohexane; (7) trivalent; (8) pentavalent; (9) structural oscillation; (10) aromatic substances; (11) valency oscillation; (12) deformation oscillation; (13) symmetrical; (14) asymmetrical.

Table 31.4 (Continued)

Frequenz (cm^{-1})	Struktur	Schwingung	Art
1140 ... 1170	$(CH_3)_2C$		Gerüstschw.
1140 ... 1160	$-SO_2-$		
1140 ... 1180	$-SO_2-N-$		
1143 ... 1163	$P-O-C_2H_5$		
1145 ... 1200	$-O-SO_2-$		
1146 ... 1149	$P-O-C_3H_7-i$		
1150 ... 1230	$-O-SO_2-O$		
1150 ... 1210	SO_3H und SO_3		
1150 ... 1163	P—O-Aryl		
1156 ... 1163	P—O-Äthyl		
1168 ... 1200	CH_3-O-P		
1165 ... 1175	$(CH_3)_2C$		Gerüstschw.
1170 ... 1310	P=O	P=O	V
1184	$P-O-C_3H_7-i$		
1187 ... 1193	$P-O-CH_3$		
1190 ... 1240	P—O-Aryl		
1200 ... 1250	$(CH_3)_3C-R$		Gerüstschw.
etwa 1228	$P-C_2H_5$		
1245 ... 1255	$(CH_3)_3C-R$		Gerüstschw.
etwa 1280	$P-CH_3$	P—C	D (12)
1310 ... 1320	$P-CH_3$		
etwa 1340	—CH—	C—H	D
1300 ... 1350	$-SO_2-$		
1300 ... 1350	$-SO_2N-$		
1330 ... 1420	$-O-SO_2$		
1350 ... 1440	$-O-SO_2-O-$		
1365	$-C-(CH_3)_3$	C—H	
1365 ... 1370	$-C-(CH_3)_2$	C—H	
1370 ... 1380	$-C-CH_3$	C—H	D sym. (13)
1380 ... 1385	$-C-(CH_3)_2$	C—H	D
1385 ... 1395	$-C-(CH_3)_3$	C—H	D
1435 ... 1450	P-Aryl		
1430 ... 1470	$-C-CH_3$	C—H	D asym.
1445 ... 1485	$-CH_2-$		
1470 ... 1500	—N—C=S		
2215 ... 2260	$P-C\equiv N$	C=N	V
2350 ... 2440	P—H	P—H	V
2525 ... 2735	P(O)OH	O—H	
2550 ... 2600	—S—H	S—H	
2560 ... 2700	P—OH	O—H	V
2843 ... 2863	CH_2	C—H	V

Frequenz (cm^{-1})	Struktur	Schwingung	Art
2862 ... 2882	CH_3	C—H	V
2880 ... 2900	CH	C—H	V
2926	CH_2	C—H	V
2962	CH_3	C—H	V
3080 ... 3160	Aromaten	C—H	V
3150 ... 3400	P—N—H	N—H	V

31.6.2. IR Spectroscopy

The infrared spectrum arises through the interaction between the electromagnetic radiation and matter, molecules and atoms. It is an absorption spectrum which arises through the conversion of radiation energy into the energy of the material particle. The energy of the infrared radiation is so small that its interaction with the material particles leads only to a change of the rotation oscillation states in the molecule and therefore to the formation of a molecule rotation oscillation spectrum. When the radiation reaches the material particle changes take place both in the rotation and the oscillation state of the molecules. In this connection some individual atoms or groups of atoms are excited which, since the transition from one rotation oscillation state to another is bound with the satisfaction of the conditions following from quantum mechanics, form as a function of the wavelength, wave number or frequency, an infrared absorption spectrum.

In recent literature the infrared radiation is characterized almost exclusively through the wave number which is defined as the number of waves occurring over a distance of 1 cm. It is related with the wavelength λ by the relation $\nu = 1/\lambda$, if λ is expressed in centimeters and has the dimensions consequently of cm-1.

The oscillations can occur on one hand as valency oscillations, that is oscillations in the direction of the bond, on the other hand as bending, twisting, pitching, and rotating oscillations. The latter is often grouped under the term deformation oscillations.

Besides these basic oscillations in the spectrum harmonics and combination oscillations also occur but they are mostly much weaker. The assumption for the indication of an oscillation is the absorption of the infrared radiation which occurs only when the oscillation is involved with the change of a dipole moment.

The great important which organic phosphorus compounds have assumed as insecticides and softeners, both in medicine, pharmacology and military chemistry suggested very thorough investigations of their IR spectra. After the assignment of the observed maxima (absorption bands) to the individual oscillations, that is the relationship between the structure and IR spectrum had been clarified, subsequently various practical problems were solved by means of the IR spectroscopy, such as the study of the hydrolisis of tabun [289] and the control of purity and study of the stability of sarin [290].

The Table 31.4. was established from many literature data. It gives in orderly sequence a survey over the position of the absorption band for organophosphoric compounds. It is striking to note that a more or less large frequency range is indicated for an oscillation. This is to attributed on one hand to the fact that different substitutes in the molecule contribute to the shift of an absorption band, on the other hand it may be due to the use of different types of equipment in the recording of the spectra.

An idea of the distribution of the absorption band should be obtained from Table 31.5 which was taken by a publication of Lorquet and Vassart [290].

To identify the substances however the assignment of the bands alone is not sufficient, only direct spectrum comparison allows a clear identification of the substance occurring.

Another possibility of applying IR spectroscopy besides identification is mainly the quantitative determination based on the Lambert-Beer law.

$$\ln \frac{I_0}{I} = \varepsilon \cdot c \cdot d = E$$

Io = radiation intensity before the test
I = radiation intensity after carrying out the test
ε = extinction module (material parameter depending on wavelength)
c = concentration (mol/l)
d = layer thickness of the dish (cm)
E = extinction

Table 31.5 IR Spectrum of Sarin

Frequenz in cm^{-1} (1)	Intensität (2)	Schwingung (3)	zugeordnete Art (4)	Gruppe (5)
720	st	P—C	sym. V (14)	P—O—C
777	m-st	P—O	V	P—O—C
840	sst	P—F	V (8)	P—F
880	schw			
905	sst (10)		K (9)	O—C_3H_7—i
925	sst			
1015	sst	O—C	V	P—O—C
1105	st (11)		K	O—C_3H_7—i
1145	m (12)		(6) Gerüstschwingung	$C(CH_3)_2$
1180	m		K	O—iC_3H_7
1280	sst	P=O	V	P=O
1320	sst	C—H	asym. K (15)	P—CH_3
1360	schw (13)	C—H	K	—C—H
1380	m			CH_3 der Isopropoxy-
1390	m			gruppe (7)
1420	m	C—H	K	
1455	m			CH_3
1470	breit			
2854	sst			
2922	schw	CH	V	—CH_3
2955	st			

Key: (1) frequency in cm-1; (2) intensity; (3) oscillation; (4) assigned type; (5) group; (6) structural oscillation; (7) CH3 of the isopropoxy group; (8) valency oscillation; (9) bend oscillation; (10) very strong; (11) strong; (12) medium; (13) weak; (14) symmetrical; (15) asymmetrical

The evaluation of the spectra using a strong and clear band can be carried out according to the baseline method, compensation method or the method of the difference in extinction. The details may be obtained in the relevant specialized literature.

To provide for deviations from the Lambert-Beer law, the work is carried out advantageously with calibration curves in which the absorption is plotted as a function of the concentration of the corresponding substance.

For the quantitative infrared spectrometric determination of organophosphoric compounds it is preferable to use the very intense absorption band at about 980 cm-1 occurring for all organic compounds of pentavalent phosphorus and which were assigned by Bellamy [291] to the P-O bond.

Because of the high reproducibility of the measurement values, the fast and clean procedure and the precision of the indications, the IR spectroscopy can be applied advantageously for reaction kinetics investigations and for determining the reaction mechanisms. The drawback is that because of the dish material and the self-absorption of the water it is impossible to work in aqueous solutions.

Section 32.3.3. reports on the field application of IR spectroscopy.

31.7. Separation and Identification by Chromatographic Methods

Chromatography is an important physical and chemical method of separation in which the substances are separated generally through the differences in the adsorption equilibria with a solid phase or through the different distribution between two liquid or one gaseous and one liquid phase. In many cases both adsorption and distribution are simultaneously effective. The distribution of the substances takes place between immobile phase and a stationery phase. The mobile or moving phases are solvent, or in the special case of gas chromatography, a carrier gas, while the stationary phases used are adsorbers or liquids fixed on a solid carrier. Special forms of chromatography are ion exchange chromatography and the separation method with molecular sifting or gel filtering. The most extensively applied method so far are column, paper and thin layer chromatography, also gas chromatography.

31.7.1. Column Chromatography

The stationary phase at which suitable adsorbers such as silica gel and aluminum oxide or cellulose powder and ion exchanges are used is in a tube. On this column first the mixture of substances to be separated is placed in a dissolved form, then the mobile phase. By adsorption or distribution spatially separate zones of the individual components of the substance are formed which are obtained either after decomposition of the column or on the basis of the different phases in time with which they emerge from the mobile phase of the column. The column chromatography allows the purification and separation of the amount of substances prepared.

The liquid column chromatography has made enormous progress in recent years. With liquid chromatography all substances which can be dissolved in suitable solvent can be analyzed. Thus less limits are imposed on the use of liquid chromatography than for gas chromatography, in which only substances which occur in the gaseous form or can be evaporated will be detectable. The development especially of the liquid column

chromatography.. But the development in particular of liquid column chromatography was limited hitherto by the want of highly sensitive and rapid indication detectors for the qualitative and quantitative measurement of the fractions leaving the column. Because of the discovery of such suitable detectors as for example the ultraviolet photometer detector, limiting concentrations up to less than 1 by 10-1 mg/ml can be detected and through the development of high pressure liquid chromatography the liquid column chromatography is assuming increasing importance. Through the increase in pressure the speed of flow of the mobile phase is increased and the time of analysis reduced considerably.

The impurities contained in technical sarin such as pyroester, hydrofluoric acid, methylphosphonic acid and other organic acids containing phosphorus can be removed by means of a chromatographic column containing water saturated silica gels [210]. A solution of sarin is allowed to pass in diisopropylether through the column, while the impurities indicated are retained and can be eluted with water after the passage of the ether solution containing sarin. The separation of various insecticides thiophosphoric esters, such as paraoxon, parathion, chlorthion and bromthion was achieved by Sandi [203] on a column of hostalene powder (low pressure polyethylene) using cyclohexane as stationary phase from a mixture of ethanol-acetate-buffer as mobile phase. To separate mixtures of different organic acids of phosphorus and their esters the displacement chromatography on cation exchanger columns proved to be suitable [211-212] and a column with anion exchanger Dowex 1-X8 was used along with hydrochloric acid as eluting agent to separate products of hydrolyisis of organo-phosphoric compounds [229].

31.7.2. Paper Chromatography

The general procedure is as follows:

A small amount of solution of the substance is applied at a marked point, the starting point at the lower end of a paper strip; the strip is then suspended with this end in a solvent containing water. The solution or working medium is sucked up by the paper and wanders over the substance spot. According to the distribution between the working medium and the stationary phase, in this case the cellulose moistened with water, the substances wander with different speed. When the working medium front has almost reached the upper end of the paper, the strip is removed from the working medium and the front is marked. If the substances separated themselves are not colored, they must be rendered visible for evaluation by spraying the chromatograms which reagent which give in the reaction with the substances separated a colored product.

The measure for the wandering speed of the substances is the Rf value[11]:

$$\text{Rf value} = \frac{\text{distance between starting point and substance spot}}{\text{distance from starting point to the solvent front}}$$

The Rf value is characteristic for the substance. But its value depends on the working medium, the type of paper and some of the conditions (temperature, saturation of the separation chamber and the paper with the solvent vapor among others) which can not always be kept constant. Therefore it is surer to allow the standard substances to pass on broader chromatograms to whose Rf value we can refer, or to use reference substances

[11] In English: "Ratio" referred to the "front".

Paper chromatography is relatively time consuming; a 40 cm long chromatogram requires according to the type of paper, working medium and development technique (ascending or descending or horizontal technique), between 6 and 20 hours.

21.7.2.1. Organophosphoric Compounds

Paper chromatography is one of the most important methods for the chemical and biochemical investigation of these compounds. From the chromatographic behavior conclusions can be drawn on a structure of these compounds, especially the type of bond of sulfur. When using a polar stationary phase and a nonpolar mobile phase compounds with sulfur in thiono bond (P=S)-, show the maximum Rf values. Sulfur in thiol bond (P-S-) reduces the Rf values. The substitution of sulfur by oxygen also reduces the Rf values.

Solvent System

Limits are imposed on the normal behavior with water as stationary phase in the application for substances which are hardly soluble in water, because of which for some organophosphoric compounds, the work is carried out with a polar solvent as stationary phase or with reversed phases, that is a nonpolar liquid is used on the paper, such as silicon or mineral oil, and a polar solvent is used as working medium. A survey of some common solvent systems may be found in Table 31.6.

Table 31.6

Organophosphoric Compound	Solvent System	Literature
VX, similar compounds and products of hydrolysis	n-butanol-ethanol-glacial acetic acid-water (8:2:2:3)	[213]
Products of hydrolysis of sarin	I. n-butanol-ethanol-glacial acetic acid-water (8:2:1:3) II. i-butanol-i-propanol-2 mNH4)H	[214]
Parathion and similar compounds	stationary phase: silicon oil mobile phase: water-ethanol-chloroform (6:10:10)	[216, 218]
Parathion, systox and isomeric compounds	stationary phase: mineral oil mobile phase: ethanol-acetone-water (1:1:2)	[217]
Isosystox and products of oxidation	toluene-acetonitrile-methanol-water (8:2:5:5)	[219]

Detection of the Organophosphoric Compounds on the Chromatogram

A generally applicable method of detection according to Hanes and Isherwood [220] is based on the transformation into phosphoromolybdic acid and its reduction to molybdinum blue according to the following procedure:

The dry chromatogram is sprayed with a solution which is prepared by mixing 5 ml 60 percent perchloric acid, 10 ml 1 normal hydrochloric acid and 25 ml 4 percent ammonium molybdate solution and filling with water up to 100 ml. The sprayed chromatogram is heated for 7 minutes at 85 degrees C. If subsequently we allow the action of an atmosphere of hydrogen sulfide or we spray with very dilute hydrochloric tin (II)-chloride solution, the organophosphates are detected as blue spots.

The detection of thiol and thionophosphoric compounds which are difficult to hydrolyze is rendered possible by previous spraying of the chromatogram with a solution of N-bromosuccinimide.

The most sensitive detection for organophosphoryls which inhibit cholineesterase in vitro, takes place through the modified biochemical methods [216]. Many of the methods were established with different substrates; the method with horse blood serum and indoxylacetate is very convenient in its execution [221]. Compounds such as parathion which inhibit only in vivo, are oxidized by treatment of the chromatogram with bromine vapor or spraying with an N-bromosuccinimide solution into in vitro inhibitors. Other suitable spraying reagents for the detection of organophosphoric poisons on paper chromatography may be found in Table 31.7.

31.7.3. Thin Layer Chromatography

The separation takes place in this chromatographic method on thin layers of adsorbers, which are on a suitable solid support, for example glass plates. The principle of separation is then mainly adsorption, but when other layers are used, distribution, ion exchange and gel filtering can also be affected. The advantages of thin layer chromatography are short working time, excellent separation sharpness, high sensitivity and general applicability because of the extensive variability of the stationary phases. The selection of the detection reagents is greater than for paper chromatography, since some aggressive reagents, such as iodine, potassium permanganate, and concentrated sulfuric acid can also be used.

The adsorbers used most frequently are fine-grained silica gel, or aluminum oxide containing a binder (gypsum or starch) and after they have been agitated with water into a paste, they are applied by means of a suitable spreading device into layers about 0.25 mm thick on the supports. The coated plates are dried subsequently. The general procedure in the development of the chromatograms is similar to that described for paper chromatography. Because of the simplicity of the execution the ascending technique is preferred, in which the plates are inserted simply in the mobile phase. With regard to the evaluation through the Rf values the same applied as was indicated in paper chromatography.

In recent years various manufacturers have brought on the market ready made materials for thin layer chromatography. The adsorbers are bound by adding suitable binders (starch, polyvinylalcohol, water glass) on rigid (glass plates) or flexible (aluminum or polyester foil) supports in tear-resistant layers. As compared with the self-coated glass plates, the industrially precoated foils have the advantage that the layers are standardized with regard to their thickness, homogeneity and their chromatographic properties. The flexible supports and the tear resistance of the layer allows the safe transport and storage of large quantities of material, which allows the application of thin layer chromatography in field analysis. Another advantage is that the ready-made foils are inscribed just like paper and can be cut to any format.

The development of chromatographs in the application of DC ready-made foil takes place preferably in the "S-separation chamber". The latter consists of a tubular tank sealed on both sides and provided with a longitudinal slit for receiving the working medium and the separation chamber frame. The frame is a plate provided on three sides with small seating shoulder. Between this frame and a second smooth plate the DC ready-made foil is clamped after applying the substances by means of a clamp and placed through the slit into the tubular tank. The advantage of the S-chamber is the low consumption of solvent even with very rapid saturation of the chamber atmosphere with the solvent vapor.

Possibilities of manifold and new applications for thin layer chromatography may be obtained by combination with the TAS method[12] developed by Stahl [286] and which corresponds basically to the method of air extraction of the warfare agents applied in the field laboratory. Many organic substances are volatile at higher temperature and can be separated thus from the specimen materials and made to undergo chromatography after direct transfer to a DC plate. Figure 31.8 shows the schematic structure of the TAS furnace.

The specimen is placed in a glass cartridge. The cartridge sealed tight at the rear end is placed in a preheated block furnace. The volatile substances evaporate and leave through the capillary opening of the cartridge as a jet of vapor. Directly in front of this opening the DC plate is held on which the volatile substances deposit at starting point and subsequently undergo chromatography. The TAS method was applied among other things already for the separation of alkaloids, herbicides, insecticide organophosphoryls and smoke poisons, including LSD.

31.7.3.1. Separation and Detection of Chemical Warfare Agents

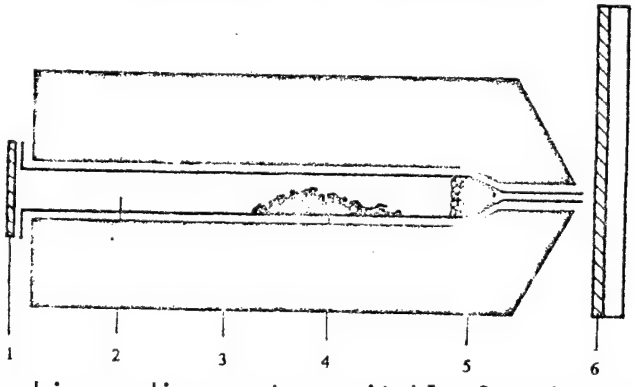


Figure 31.8: TAS Furnace
Key: (1) seal; (2) glass cartridge; (3) heating block (furnace); (4) specimen; (5) glass wall; (6) DC layer.

A working medium system suitable for the separation of DFP, tabun, sarin and soman on silica gel layers is n-hexane-acetone-methylenechloride (7:2:1) [187]. The Rf values of the warfare agents are for DFP 0.5, tabun 0.26, sarin 0.27 and for soman 0.41. A mixture of n-hexane and ethylacetate in the proportions 3:1 and with n-hexane-acetone (4:1) most of the thiolorganophosphoryls can be separated on silica gel layers. To separate organophosphoric warfare agents of the type VX with a tertiary nitrogen atom in the molecule, a small amount of a base, for example diethylamine must be added to the working media in order to prevent retention at the starting point caused by salt formation on the weakly acid silica gel layers. For

[12] TAS = abbreviation for thermomicro-, separating, transfer and application method for substances according to Stahl.

the simultaneous separation of warfare agents of the sarin type such basic working media are not suitable however, since the amines would destroy these agents.

The maximum sensitivity for the detection of the organophosphoric warfare agents on the chromatograms was found in the biochemical methods based on the inhibition of choline and aliesterases.

The procedure with indoxylacetate as substrate offers the advantage that the inhibitor spots can be identified in the UV light as well as in daylight /221/.

Spray solutions:

A. Enzyme solution (equal parts of a 10 percent aqueous solution of lyophilized horse blood serum and testal standard buffer of pH 10.10 are mixed).

B. Substrate solution (0.5 percent indoxylacetate are dissolved in a mixture of 2 parts of acetone free from aldehyde and 3 parts ammonia).

Procedure:

The plates taken from the separation chamber are moistened after evaporation of the working medium by spraying uniformly with the enzyme solution and subsequently kept for 10 minutes (for sarin and soman) or 30 minutes (for VX) in a vessel with atmosphere saturated with water vapor. After the incubation period the plates are sprayed with a substrate solution and examined in the light of an ultraviolet lamp. After 1 to 2 minutes the inhibitors are identified as dark spots against a strong bluish-green fluorescent background. With the gradual disappearance of the fluorescence then by the oxidation of air of indoxyl into indigo a blue basic color appears, against which the inhibitor spots appear white. The detection limit is 0.005 µg sarin and soman and 0.05 µg VX. For the exclusive evaluation in ultraviolet light the use of n-methylindoxylacetate is advantageous at substrate /197/, since the fluorescence lasts longer. The detection sensitivity is higher in ultraviolet evaluation but when used on extracts of different specimen material disturbances appear more strongly from the contents of the specimens extinguishing fluorescence and extracted at the same time.

More accessible than horse blood serum esterase are aliesterase preparations of cattle liver /196/ so that we will describe their application for the biochemical detection of organophosphoric warfare agents on thin layer chromatograms.

Spray solutions:

A. Enzyme solution (1 part cattle liver is homogenized with 5 parts of water. If no homogenizer is available, the liver cut into small pieces can be crushed thoroughly in the mortar with sea sand and then mixed with water. The homogenisate is centrifuged for 10 minutes at 5000 RPM. The supernatant solution is mixed with testal standard buffer of pH 10.0 in the proportion 1:1 and used as spray solution.

   The homogenisate can be freeze dried under vacuum. The dry preparation obtained retains its activity for years if it is kept cool and protected from humidity. To produce a spray solution 20 mg are dissolved in 10 ml buffer.

B. Substrate solution (20 mg α-naphthylacetate are dissolved in 2 ml acetone free from aldehyde; 6 ml water are added).

C. Coupling reagent (10 mg fast blue salt[13] are dissolved in 8 ml water).

Procedure:

General procedure is similar to the one described in the biochemical detection with ChE and indoxylacetate. After the incubation time the chromatograms are sprayed one after the other with the substrate solution and the coupling reagent. The inhibitor spots are recognizable after a few minutes white against a violet background.

A survey of other spray reagents, especially for the detection of organophosphoric poisons containing sulfur may be found in Table 31.7.

The general detection method described in 31.7.2.1. through the transformation into phosphoromolybdic acid can also be applied to detect organophosphoric poisons on thin layer chromatograms. After spraying the chromatograms are heated for 10 minutes at 110 degrees C.

Yperites

The solvent mixture of chloroform and acetone (5:4) is suitable for the separation of sulfur yperite and its products of hydrolysis on silica gel layers /191/.

Sulfur and nitrogen yperites can be detected with high sensitivity on thin layer chromatograms with 4-(p-nitrobenzyl)-pyridine.

Spray solutions /198/:

A. 2 percent solution of 4-(p-nitrobenzyl)-pyridine in acetone.

B. 10 percent solution of ammonium carbonate in a mixture of water and acetone (1:1)

C. 10 percent potassium hydroxide solution in 50 percent ethanol.

Procedure:

After the evaporation of the working medium the chromatogram is sprayed strongly with spray solution A, then slightly with B and subsequently heated for 10 minutes at 110 to 120 degrees C. After cooling it is sprayed with a spray solution C. The yperite as well as the irritant chloracetophenone are visible as blue spots. The detection limit is 1 µg.

Sulfur yperite can be detected on silica gel layers, containing fluoresceine by treatment with bromine vapor. The color of the background is represented by the red tetrabromofluoresceine (eosine) to which the yperite spots, in which the bromine is transformed by oxidation of the yperite into sulfoxide, gives a yellow contrast.

[13]Double zinc salt of tetraazotized o-dianisidine.

In ultraviolet light the yperite spots can be recognized by a yellow fluorescence on a dark background. The detection limit is 5 g sulfur yperite. The layers containing fluoresceine are obtained by agitating the silica gel to coat the plates with water, which contains dissolved 0.04 percent sodium fluoresceine.

Nitrogen yperites can after spraying of the chromatograms with the modified Dragendorff reagent be identified by the orange-red color of the complex compound described in section 28.3.11.

Table 31.7

Poison	Spray Reagent, Procedure, Sensitivity	Literature
DFP, tabun, sarin, soman	5 percent aqueous NaOH containing 1 percent H_2O_2; then dried at 110 degrees C, sprayed with saturated solution of dehydrated $CoCl_2$ in acetone; dry again at 110 degrees C blue spots on white background detection limits: 10 g sarin and soman, 20 µg DFP, 40 µg tabun.	[187]
organophosphoryls containing sulfur (among others VX)	a) 5 percent palladiumchloride in 2 ml hydrochloric acid and 98 ml water →according to the type of sulfur bond yellow (for -S-C, -SO-C and -SO2-C) to brown (for P S and -SH) spots detection limits: 2-10 µg	[222]
	b) potassiumhexaiodoplatinate 1 g platinumchloride dissolved in 10 ml water and mixed with a solution of 10 g KI mixed in 250 ml water, before it is used it is diluted 1:6 with water. → yellow spots on pink background detection limit: 1 - 5 µg.	[215]
	c) 5 percent NaN_3 in 0.1 N iodine solution → white spots on brown background detection limit: 1 to 10 µg	[223]
organophosphoryls containing sulfur with P S groups	0.5 percent 2,6-dibromo-p-benzoquinone-4-chlorimide in cyclohexanol; heat 5 minutes at 110 degrees C →red spots detection limits: 0.1 to 0.2 µg	[224]
organophosphoryls with tertiary aminonitrogen or a choline group (tabun, VX)	Modified Dragendorff reagent Solution A: 0.85 g basic bismuth nitrate are dissolved in 10 ml glacial acetic acid and 40 ml water Solution B: 8 g potassium iodide are dissolved in 20 ml water. Stock solution: Equal volume parts of solution A and B are mixed. Spray solution: For use 1 ml stock solution is mixed with 2 ml glacial acetic acid and 10 ml water. →orange spots on yellowish background. detection limits: 2 - 5 µg.	[195]

Table 31.7 (continued)

Poison	Spray Reagent, Procedure, Sensitivity	Literature
thiolphosphonates (VX)	0.2 N NaOH in 50 percent ethanol, after a few minutes with 1 percent 5,5'-dithiobis(2-nitrobenzoic acid) in mixture of 1 part ethanol and 1 part 0.45 M tris-buffer of pH 8.2 → yellow spots.	/186/

Other Warfare Agents

Bromobenzylcyanide (CA), chlracetophenone (CN) and 2-chlorobenzylidenemalodinitrile (CS) can be separated on layers of acid aluminum oxide with the mixture of solvents benzol and chloroform (19:1). Benzol-dichloromethane (3:1) is suitable to separate chloracetophenone an 2-chlorobenzylidenmalodinitrile with secondary products on the same layers /188/.

The irritating agents indicated are detected by spraying the chromatograms with a 0.5 percent solution of p-benzoquinone in methanol, subsequently if 1:1 diluted ammonium solution through a brown (CN), yellow (CA) or blue (CS) spot /188/. The detection limit is 5 to 10 µg. With the detection system described in 31.7.3.1. with 4-(p-nitrobenzyl)-pyridine CS gives a yellowish-orange, CN gives a blue and CA a reddish violet spot.

Adamsite is detected with a red spot when the chromatograms are sprayed with concentrated sulfuric acid. The same spray reagent is also suitable for the detection of the psychotoxic warfare agent BZ. When the chromatograms are heated to 110 degrees C BZ and the other benzilic esters give carmine red spots.

Potassiumhexaiodoplatinate or a serium sulfate-sulfuric acid reagent (→ red spots) can also be used to detect BZ. The separation takes place on silica gel-G layers with the medium cyclohexane-chloroform-diethylamine (5:4:1) or methanol-ammonia solution (99:1) /189/.

LSD and related indole substances can be separated on silica gel G layers with chloroform methanol (9:1) as working medium /231/. The detection takes place in ultraviolet light (360 and 254 nm) by observing the blue fluorescence or by spraying with a modified Van Urk reagent (0.5 g p-dimethylaminobenzaldehyde dissolved in 95 ml ethanol and 5 ml concentrated hydrochloric acid). The reagent indicates the presence of these compounds by a blue-violet color. The detection limit is 0.05 µg LSD /190/.

31.7.3.2. Sabotage Poisons and Herbicides

Sodiumfluoracetate can be separated on silica gel G layers with a working medium containing 4 percent ammonia in ethanol. The detection takes place by spraying with alcoholic silver nitrate solution and irradiation with ultraviolet light of wavelength 360 nm. Fluorine acetate is identified up to a detection limit of 0.1 µg by a dark spot /192/.

Various chlorinated phenoxylcarbonic acids, such as 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxybutyric acid (2-4-DB), 4-chloro-2-methyl-phenoxyacetic acid (MCPA) and α,α-dichloropropionic acid (dalapon) can be separated on layers which consist of 60 percent kieselguhr and 40 percent silica gel with starch as binder. The working medium used is paraffin oil-benzol-glacial acetic acid-cyclohexane (1:3:2:20); the detection takes place by spraying with 0.5 percent alcohol silver nitrate solution and irradiation with ultraviolet light, while brownish-black spots are visible against a white background [230].

After the separation on the ready-made foil silufol UV 254 (the manufacturer is the Cavalir Company, Prague, Czechoslavakia) with the working medium benzol-acetone (40:60) these herbicides can be detected by spraying with a solution of 0.02 percent rhodamine B in ethanol and 5 minutes irradiation with ultraviolet light, which makes them visible as violet spots against a pink background [193].

The detection of the chlorinated phenoxy-fatty acids after the separation on silica gel-G layers by spraying with a reagent which consists of a solution of 4 g sodium salt of chromotropic acid in 40 g water and 56 g sulfuric acid. When the chromatograms are heated to 160 degrees C the herbicides are visible as violet spots against a white background [199].

31.7.3.3. Alkaloids

The working media used are:

Number	Working Medium	Reference Substance	Rf
I	chloroform-acetone-diethylamine (50:40:10)	rhodamine B	0.58
II	chloroform-diethylamine (90:10)	rhodamine B	0.49
III	cyclohexane-chloroform-diethylamine (50:40:10)	rhodamine B	0.20
IV	cyclohexane-diethylamine (90:10)	butter yellow	0.45
V	benzol-ethylacetate-diethylamine (70:20:10)	butter yellow	0.44
VI	chloroform	butter yellow	0.85
VII	cyclohexane-chloroform (30:70) + 3 drops diethylamine	butter yellow	0.85
VIII	methanol	rhodamine B	0.53

The thin layer chromatography allows the systematic analysis of the alkaloids according to a procedure established by Waldi, Schnackerz and Munter [227]. In this connection the alkaloids are subdivided into 2 groups and we establish first through a preliminary testing with the working medium 3 on silica gel G layer which is the group of the unknown substance. The evaluation takes place by examination in the ultraviolet light and by spraying with potassium hexaiodoplatinate reagent. The reference substance used is rhodamine B. Alkaloids with a Rf value below 0.30 are then assigned to

Group I, those with Rf values above 0.30 to Group II. The identification of Group I then takes place besides the evaluation of color reactions by separation with the working media I and II on silica gel layers, those of Group II by separation with the media III, IV and V also on silica gel. In cases of doubt the separation can also be carried out with the media VI, VII, and VIII on aluminum oxide G (D) and alkalized silica gel (instead of water 0.1 normal sodium hydroxide is used to stir in the coating substance). The most favorable amount applied is 50 g substance to be studied.

The evaluation takes place according to Table 31.8.

The separation of a wide spectrum of toxic compounds including among others the alkaloids is possible with a working medium which contains methanol/acetone/triethanolamine (50:50:1.5) on silica gel layers [228]. A previous separation according to the Stas-Otto method is proper.

Table 31.8. Rf Values and Color Reactions of Alkaloids

Layer	K	K	K	K	K	A	A	K (alk.)	Fluorescence Color in Ultraviolet (365 μm)	Color with Iodo-platinate reagent	Form of the Spot	Number of Secondary Spots
Working Medium	I	II	III	IV	V	VI	VII	VIII				
						Group I						
morphine	0.10	0.08	0.00	0.00	0.03	0.03	0.00	0.34	--	deep blue	round	--
atropine	0.38	0.40	0.16	0.05	0.12	0.00	0.10	0.17	--	violet blue	round	--
colchizine	0.47	0.41	0.04	0.00	0.04	0.11	0.00	0.57	--	light gray	round	--
homatropine	0.37	0.45	0.15	0.05	0.23	0.04	0.24	0.15	--	violet blue	round	--
brucine	0.42	0.63	0.18	0.00	0.19	0.50	0.54	0.12	--	violet brown	round	1
strychnine	0.53	0.76	0.28	0.05	0.38	0.57	0.60	0.22	--	yellow	drawn	--
						Group II						
physostigmine	0.65	0.90	0.32	0.04	0.44	0.59	0.50	0.46	--	pink	round	1
aconitine	0.68	0.90	0.35	0.03	0.49	0.36	0.60	0.65	--	reddish brown	round	3
emetin	0.67	0.90	0.40	0.06	0.45	0.38	0.58	0.50	blue	reddish brown	round	3
papaverine	0.67	0.90	0.42	0.03	0.47	0.85	0.84	0.70	yellowish	yellow	round	--
narcotine	0.72	0.90	0.51	0.10	0.57	0.81	0.79	0.72	blue	light yellow	round	--
cocaine	0.73	0.90	0.65	0.36	0.58	0.84	0.77	0.62	--	violet	round	--

K = silica gel; A = aluminum oxide; K (alk) = alkaline silica gel

31.7.4 Gas Chromatography

Gas chromatography is one of the latest chromatographic techniques but over the two decades which have elapsed since the beginning of its whirlwind development, it has

become one of the most important methods of organic and chemical analysis and new areas of application are constantly being open to it. Its special advantage as compared with the previously discussed chromatographic methods is the possibility of recording the separation possible to record simultaneously by a simple method the separation process both qualitatively and quantitatively. Gas chromatography is similar to column chromatography, but instead of the liquid medium a gaseous mobile phase is used, the carrier or extracting gas. The area of application is thus fixed for gaseous substances or specimens which can evaporated without destruction, but characteristic volatile products of pyrolysis can also be separated by gas chromatography and information obtained on the composition of the specimens. The gas or vapor specimen is fed to the carrier gas current; the latter passes through a tube which can be tempered and is filled with a stationary phase. The stationary phase used is a solid adsorber such as silica gel or active carbon or a liquid of low vapor pressure which is placed on an inert, porous carrier material. Besides the filled columns recently capillary columns have been used which have capillaries with an internal diameter of 0.2 to 0.5 mm, but are up to 150 m long, on whose internal wall a liquid film forms the stationary phase. The separating effect of these capillary columns is extremely high; we may expect theoretically 100,000 to 500,000 plates, since a good distillation column has about 100 theoretical plates. Using short capillary columns, extremely short times of analysis are reached which often amount to only fractions of a second; naturally the cost of the equipment is really high.

The indication of the components appearing after passing through the separation columns at intervals in the carrier gas current is carried out with detectors which are mostly based on physical principles such as measurement of thermal conductivity and gas density, flame and radiation ionization, or mass spectrometry and whose indication is registered with an automatic recorder. Recording curves are obtained with a number of tips (peaks) expressing the number of components, and whose distances from a starting point represent the retention times important for the qualitative evaluation. The peak areas are used for quantitative evaluation.

Gas chromatography allows the analysis of complicated mixtures in a fraction of the time needed for the ordinary methods and is one of the most sensitive methods to detect and determine toxic substances.

31.7.4.1. Organophosphoric Compounds

Whereas silicone oils and high vacuum fats are used very generally as stationary phases and rough-grained kieselguhr (chromosorb) as carrier material, the use of gas chromatography for separating and determining organophosphoric compounds led to some very interesting new developments for detectors.

Of the ordinary detectors, the thermal conductivity measurement cell and the flame ionization detectors are suitable for indicating the organophosphoric compound. For the very sensitive detection of compounds with high electron affinity, including the insecticides and herbicides containing chlorine and various toxic insecticides, organophosphoric compounds, a modification of the radiation ionization detector, the so-called electron accumulation or electron catcher detector has assumed great importance.

Through the beta irradiation of a tritium source the molecules of the nitrogen used as carrier gas are ionized, free electrons are formed which under the effect of a fixed

potential applied to the detector cell wander to the anode acting as measurement electrode and give a stable outlet current. If electron accumulating molecules occur in the specimen substance, negative ions are formed which move more slowly in the electrical field and almost all combine with positive ions before they reach the anode. The ionization currents measured initially with the pure carrier gas is reduced by an amount proportional to the number of the negative ions formed per unity of time. Up to a certain level there is proportionality between the signal of this type of detector and the product of the concentration and electron accumulation capacity of the compound. The different incorporation capacities measurable with the electron accumulation detector is suitable for identifying unknown compounds. The average sensitivity of the detector for organophosphoric compounds is in the nanogram range.

An arrangement in which the compounds contained in the carrier gas current after passage through the separating column is burned in a quartz tube in an oxygen current or reduced by hydrogen at high temperatures is widely used to determine chlorine, sulfur, and phosphorus compounds; the reaction products are then determined continuously in a coulometric titration cell. As compared with the electron catcher detectors in these the method has a lower sensitivity, but the indication is element-specific and is less subject to disturbances by other organic compounds. It is therefore very suitable to determine organophosphoryls in the most different specimen materials. Concentrations of 25 to 50 ng/l water could be determined in association with a method of extraction according to this technique [233].

The specific indication of phosphoric compounds at higher sensitivity is allowed with the sodium thermionic detector which was described for the first time in 1964 by L. Giuffrida [234]. It consists of a wire coil drawn with a sodium soil, which is placed directly over the normal hydrogen-flame ionization detector. The salt heated by the flame gives an ionic current which in the passage of organic halogen and phosphorus compounds is greatly amplified by the detector.

This arrangement was improved subsequently several times and in the modern variants of this detector the wire coil drawn with alkaline salt is replaced by a pierced salt tip placed on the burner of the flame ionization detector and which is produced as compressed cesium bromide or rubidium sulfate. Organophosphoric compounds are detected with this detector in picogram (10-12 g), compounds containing chlorine in the nanogram (10-9 g) amounts.

A high specificity of the indication is achieved by combination of gas chromatography with emission spectrometry. In the upper part of the hydrogen flame of the flame ionization detector the intensity of the emission bands of phosphorus (256 nm) and sulfur (394 nm) present in the passage of poison is measured with a sensitive photometer. The detection limit is 10 ng parathion.

It is apparent from the above short description of the individual types of detectors, that the development tends toward an increase in specificity of the indication. The method of gas chromatography is specifically the most universal method of separation of complicated gas mixtures, but the qualitative characterization of the components with the highly sensitive detectors but with nonspecific indications raises considerable difficulties and is mostly possible only by comparison with standard substances. Since on the other hand the technique of mass spectrometry allows the exact identification of

organic compounds with extraordinarily low amount of specimen, but the direct analysis of a complex mixture of unknown components by this method raises almost insoluble problems, the logical consequence of the previous development was to combine the gas chromatographic separation with mass spectrometric identification. Since with amounts of specimen of only 10-8 to 10-10 g it is possible to obtain mass spectra which can still be interpreted, the detection limit achievable by combining the gas chromatograph with mass spectrometer is in order of magnitude in the range of indication of the sensitive flame ionization detector. The combination can be obtained both directly for the continuous operation and discontinuously, by collecting the components separated in the gas chromatograph individually in the adsorption tube and later undergoing desorption in a mass spectrometer. Since for this procedure the spatial separation of gas chromatograph and mass spectrometer is possible, it was possible to examine centrally specimens from different laboratories with a mass spectrometer of particularly high quality.

To study the organophosphoric compounds of high boiling points which can be evaporated without destruction, it is possible to use the products of pyrolysis, the alkyliodides after carrying out the Zeisel alkoxyl reaction, the methylester after treating with diazomethane or especially for systox and similar compounds, the volatile thioether after hydrolysis with alkalides or alcoholates. The combination of thin layer chromatography with gas chromatography allows the separation of mixtures of substances containing components which cannot be distilled.

Examples of the application of the gas chromatography method for organophosphoric poisons are the separation obtained by Verweli et al of diastereoisomeric O-alkylmethyl fluorophosphonates and similar pyrophosphonic esters and the direct conversion of soman with antidotes followed by Okonek [237] by means of gas chromatography. This author used a glass column 1.5 m long with a diameter of 2 mm, which was filled with 5 percent SE-30 (silicone) and 5 percent QF-1 (fluoralkylsilicone) on chromosorb W-AW-DMCS (kieselguhr washed with acid and treated with dimethylchlorsilane) 100/120 mesh. The temperature of the column was 130 degrees C, the indications of the soman was obtained with an alkali thermionic detector.

31.7.4.2. Other Warfare Agents and Poisons

Sulfur yperite and its product of hydrolysis were separated in a column of 1.5 meters length which was filled with 3 percent cyclohexanedimethanolsuccinate on gas-chromium Q (100/120 mesh) at a column temperature of 120 degrees. The indication was achieved with a flame ionization detector, while in the case of sulfur yperite a linear relation was established between the amount of specimen and the peak area up to 50 g yperite [238].

The indication of sulfur yperite is more specific with a flame photometric detector which measures by means of a suitable filter the intensity of the emission band of sulfur at 394 nm. The gas chromatographic separation takes place with 4 percent FFAP on chromosorb W AW-DMCS (60 to 80 mesh) for a column temperature of 155 degrees C. When adding 1 g specimen solution, the limiting concentration is 2 by 10-4 mg/ml and the calibration curve is linear in the region of 1 to 120 µg/ml [322].

Bromobenzylcyanide (CA), 2-chlorobenzilidenemalonodinitrile (CS) and chloracetophenone (CN) were separated on 10 percent QF-1 on gas chromium Q (60/80 mesh) in a column of 1.5 m length at temperatures of 65 to 200 degrees C. With the thermal conductivity detector it is possible to indicate amounts in the milligram range, with the flame ionization detector microgram and with the electron catcher detector levels in the nanogram range. The limiting concentrations achievable with the electron catcher detector for the study of contaminated air were 0.4 mg CA/m3, 0.02 mg CN/m3 and 0.01 mg CS/m3 /239/.

The determination of phosgene in the air up to a limiting concentration of 1 by 10-4 mg/l can be obtained by gas chromatographic separation and indication with the electron catcher detector /235/.

A glass column 0.80 long filled with 0.25 percent SE-30 microglass pearls is suitable for the separation of LSD. The temperature of the column should be 255 degrees C; the indication is accomplished with a flame ionization detector /240/.

The gas chromatographic separation of the chlorinated phenoxyacetic acid herbicides is possible after treatment of the extracts with diazomethane while the volatile methylesters are formed /312/. The extractions of these herbicides takes place at pH 2.0 by extraction with benzol or chloroform. While 1 liter of the specimen is treated successively with 80, 50, 30, 30 ml solvent. The yield is 35 to 80 percent for concentrations of 1 mg/l, 70 to 100 percent for concentrations above 5 ml/l /313/. Picloran is extracted from soil and plant samples with potassium hydroxide, converted after acidulation into ethylacetate and is also converted by treatment with diazomethane into the methylester volatile without destruction. The latter is determined by gas chromatography on 2.5 percent neopentylglycoladipate on silanized chromosorb W (80 to 100 mesh) with nitrogen as carrier gas at 185 degrees C using an electron catcher detector /314/. Picloran can be extracted from water and soil samples after the acidulation with acetone. 2 µl of the extract are decarboxylized in a pyrolysis section connected in front of the separation column at 385 degrees C into 4-amino-2, 3,5-trichloropyridine. The gas chromatographic separation of the reaction product takes place at 165 degrees C on a column 105 cm long with 3 percent SE-30 of chromosorb W (60-80 mesh) with a subsequent column 60 cm long with 10 percent DC-200 on gas chromium Q (60-80 mesh). The carrier gas used is nitrogen (10 ml/min); the indication is obtained with an electron catcher detector. In water specimens 5 by 10-4 to 10-1 mg/l can be detected, in salt specimens, 5 by 10-2 to 1 mg/kg /315/. The gas chromatographic separation of the bromacil extracted from plant, soil and water specimens takes place at 200 degrees C with 3 percent QF-1 fluorsilicone and 2 percent DC-200 silicone on gas chromium Q (80-100 mesh). The carrier gas used is nitrogen (30 l/min); the indication is obtained with the electron catcher detector /316/.

The separation and enrichment of 2,4-D and 2,4,5-T from water can be achieved very elegantly and quantitatively by means of a tube filled with the adsorber resin XAD-2 (divinylstyrol polymer) at pH 2.0. The dimensions of the adsorption tube are 10 by 60 mm; after the passage of 2 l water drying is carried out with a nitrogen current and subsequently the herbicides are eluted with 20 ml acetone. For the esterification 1 ml of the elute is treated with 1 ml of a 14 percent solution of boron trifluoride at 80 degrees C. After adding 5 ml water the ester is extracted with 10 ml cyclohexane. 2 µl of the extract are sprayed into the gas chromatograph. The separation takes place

on a 1 mm glass column with 2 percent OV-225 on chromosorb W-HP (100-120 mesh) at 190 degrees C. The carrier gas used is argon with 5 percent methane (50 ml/min); the indication is accomplished with an electron catcher detector. With this procedure 0.025 mg/l can still be determined [304].

Monuron and diuron are saponified in the extracts with 20 percent sodium hydroxide into the corresponding substituted anilines. The determination is achieved with temperature program gas chromatography on 100 by 0.6 cm column with 20 percent apiezone fat L on chromosorb W (60-80 mesh). The initial temperature is 75 degrees C, the rate of increase 8.8 degrees C/min. The carrier gas is helium (50 ml/min). 4-chloraniline emerges from the column at 180 degrees C, 3,4-dichloraniline at 212 degrees C. The indication is achieved with a flame ionization detector [321].

31.8. Electrochemical Methods

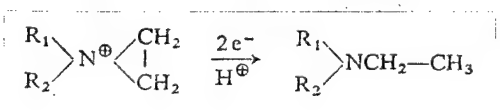
The polarographic methods with which sensitive determinations and kinetic investigations are possible and which are suitable for combination with a separation method, such as column or gas chromatography are of particular interest. Other electrochemical methods, such as potentiometric titration are indicated each time in the description of the chemical, biochemical or elementary analysis study of the warfare agents.

31.8.1. Polarographic Methods

Since in general only such ions or molecules of the polarographic determination are accessible which can be reduced or oxidized on an electrode under electron catcher or release, limits are imposed on the application of this electrochemical method to organic compounds. Some warfare agents however are accessible to polarographic determination.

Sulfur yperite is polarographically inactive against the mercury drop electrode, but for a potential of +0.9 V against the saturated calomel electrode on the rotating platinum electrode, with which we must work in this voltage range it is oxidized and can be determined quantitatively from the stage height. In the studies on catalytic waves by which we mean the catalytic shift of the hydrogen separation to a lower potential by polarographically otherwise inactive substances, Brdicka established that sulfur yperite in the presence of ammonia cobalt chloride solutions also shows this effect. This observed catalytical effect is not attributed to the sulfur yperite itself but to a reaction product with ammonia. Of the possible compounds among others, β-aminomethylmercaptane was studied polarographically and proved to be catalytically effective [207]. The quantitative determination of sulfur yperite is possible by measuring the catalytical waves following the cobalt stage.

The nitrogen yperites are polarographically inactive, but the quarternary nitrogen can be reduced polarographically in the intermediary ethyleneimonium ion formed in the hydrolysis by cyclization, while the following mechanism was postulated [208].



The polarographic method is therefore very suitable for the kinetic study of the conversion of nitrogen yperite into ethyleimonium compounds. The detection and determination of warfare agents containing arsenic, such as diphenylchloro- and cyanoarsine, adamsite and α-lewisite are possible both with the classical polarography with mercury electrode and with the oscillographic polarography [209]; in the latter, these warfare agents can be differentiated qualitatively by the different position of the sections on the two branches of the oscillogram. The application of the classical polarigraphy to the determination of the organophosphoric compound is limited, apart from the indirect methods to those which contain reduceable functional groups such as paraoxon and parathion, or in the hydrolytic separation of polarographically active products. The latter include malathion, in which the fumaric acid is measured and systox and tinox, which can be determined by measuring the catalytical hydrogen waves which are produced by the hydrolytically formed thiol groups. An important advance was achieved by oscillographic polarography with which the organophosphoryls which are inactive in classical polarography can be detected and determined with high sensitivity.

Check Questions

1. How can an unknown substance be identified by determining the melting point?

2. What is the importance of the index of refraction for characterization of organic compounds?

3. How can the infrared spectroscopy be used for the detection and determination of organophosphoric warfare agents?

4. What chromatographic methods are suitable in particular for analysis of warfare agents? Compare the methods with each other.

5. Define the Rf value and explain its importance.

6. How are biochemical methods used for the detection of the organophosphoric warfare agents by chromatography?

7. Compare the gas chromatography with regard to advantages and drawbacks with other chromatographic methods.

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SCIENTIFIC RESEARCH IN ANTARCTIC DESCRIBED

Warsaw NAUKA POLSKA in Polish No 11-12, Nov-Dec 81

[Article by Krzysztof Birkenmajer: "The Fifth Antarctic Expedition of the Polish Academy of Sciences at the H. Arctowski Station"]

[Text] The tasks of the Fifth PAN [Polish Academy of Sciences] Scientific Expedition at the Henryk Arctowski Antarctic Station, located on King George Island in the South Shetland Archipelago (West Antarctic), was to:

--complete the research and organizational cycle of the 1976-1980 5-year plan, MR.II.16: "The Integrated Investigation of the Antarctic and Arctic as a Basis To Protect and Examine Their Natural Environments;"

--initiate new investigations for the new 1981-1985 5-year cycle, MR.II.16: "Investigating the Sea and Land Polar Regions as a Basis for the Rational Use of Their Resources and the Protection of Their Environment."

The PAN Institute of Ecology, located in Dziekanow Lesny near Warsaw, organized the expedition. The administration consisted of four people: Prof Dr Eng Krzysztof Birkenmajer, expedition director; Eng Lech Rosciszewski, deputy director for general affairs (subsequently designated director of the winter group); Eng Maciej Zawadzki, deputy director for technical affairs; and Eng Karol Teliga, director of the winter group (during the expedition's organizational phase in Poland). In Poland, preparations for the expedition took about 1 year, from the fall of 1979 to the fall of 1980.

Twenty people from the summer group and 20 people from the winter group (24 people from the scientific team and 16 people from the technical team) were supposed to be included in the expedition. However, because it was necessary to reduce expedition expenditures significantly, only 24 expedition participants, including 5 people from the winter group, sailed from Szczecin on 27 November 1980 to the Arctowski Station on the first voyage of the M/S "Zulawy," a Transocean PPURM [expansion unknown] transport-supply ship commanded by Navy Captain Leon Skelnik.

The remaining member of the Fifth Expedition winter group were supposed to sail to the Arctowski Station in mid-March 1981 on a ship which in its return voyage to Poland was supposed to pick up the summer group. However, in view

of the unsuccessful attempt to evacuate some of the Fourth Expedition winter group with the aid of the fishing ship M/T Levanter during the last quarter of 1980, the entire Fifth Expedition winter group was included in the expanded Fifth expedition summer group up to the beginning of February 1981 when the S/Y "Poria," chartered by the Institute of Ecology and commanded by Navy Capt Krzysztof Baranowski, arrived at the Arctowski Station. Two members of the Fifth Expedition winter group arrived on this yatch and eight participants of the previous winter expedition (Fourth) departed on it.

Five foreigners (two from the FRG and three from the United States) also participated in the summer expedition.

Organizational Scheme of the Summer Expedition

As a result of the repeated changes in the personnel status of the Fifth Expedition mentioned above, a total of 51 people participated in the Fifth Expedition during the 1980-1981 Antarctic summer season, of which 7 remained for the winter together with 12 people who arrived at the Station on 23 March 1981 on the training-cargo ship M/S "Antoni Garnuszewski," commanded by Navy Capt T. Draczkowski.

During the summer expedition to the Arctowski Station (8 January 1981 - 27 March 1981), all station personnel were directed by a three-member management team consisting of Prof Dr K. Birkenmajer, director; Eng L. Rosciszewski, deputy director for organizational affairs; and Eng M. Rzepecki, deputy director for technical affairs. The expedition was divided into six groups of which five were scientific groups having appropriate directors and whom in essence, operated independently:

I. The Geological Photography Group: Prof K. Berkenmajer (director), W. Danowski and Eng K. Rolnicki;

II. The Sedimentology-Paleontology Group: Prof R. Gradzinski (director), Dr A. Gazdzicki, Dr S. Porebski and Dr R. Wrona;

III. The Geophysics Group: A. Szymanski (director), Eng T. Radomyski, Eng J. Komorowski (up to 5 February 1981, he sailed on the "Pogoria") and J. Weiss;

IV. The Meteorology-Oceanographic Group: Eng E. Moczydlowski (director up to 5 February 1981, he sailed on the "Pogoria"), Z. Rawa (director from 5 February 1981), Eng A. Maciazek, Eng G. Marczak and R. Marks;

V. The Biology Group: Prof E. Kolakowski (director), R. Gutkowski (to 5 February 1981, he departed on the "Pogoria"), Dr R. Halba (to 5 February 1981, he departed on the "Pogoria"), Dr S. Misiaszek, he arrived on the "Pogoria"), Dr Med W. Schramm (J. w.) [expansion unknown], Dr W. Trivelpiece (United States, from 12 February to 2 March 1981), Dr N. Volkman (j.w.), Dr R. Butler (j.w.);

VI. The Technical Group directed by Eng L. Rosciszewski, consisted of three separate teams:

VIA (the aviation team): Eng J. Grzybowski (director), Pilot E. Bienaszewicz, Pilot W. Kurzynski, Mechanic Z. Musialski and Mechanic Z. Sygit;

VIB (the maritime team): Eng M. Rzepecki (director), A. Cieslak (fisherman, cutter service), J. Kaminski (mechanic), J. Kaszubowski (PTS [expansion unknown] amphibian service up to 5 February 1981, he departed on the "Pogoria"), H. Nowak (radio operator), Eng P. Majewski (power engineer) and L. Wisniewski (PTS amphibian service);

VIC (the technical team): K. Potrzebowski (director), C. Augustyn (cook), J. Berendowski (radio operator), W. Chudzynski (electrician to 5 February 1981, he departed on the "Pogoria"), J. Krasakiewicz (mechanic), Dr Med W. Nurkiewicz (doctor), J. Styczynski (mechanic), J. Szylak-Szydlowski (Radio operator to 5 February 1981, he departed on the "Pogoria").

Expedition Program

The Scope of Scientific Work. During the Fifth Expedition the scientific research program stipulated work on all three subproblems of the MR.II.16 (currently MR.I.29) plan for the 1981-1985 period, that is: (A) the biological sciences, (B) the earth sciences and (C) oceanography. Because the primary biology program of the 1980-1981 Antarctic summer season was supposed to be realized by a separate sea expedition organized by the PAN and the Fishing Institute in the framework of the international FIBEX (BIOMASS) program on the ship R/V "Professor Siedlecki," it was projected that research at the Arctowski Station would not be as extensive as in previous years. However, research in the earth sciences--geology, paleontology and applied geophysics, especially with the aid of helicopters, was supposed to be intensified and expanded.

Personnel from the following institutions realized the Fifth Expedition scientific program: the PAN Institute of Ecology, the PAN Institute of Geological Sciences, the PAN Institute of Geophysics, the PAN Department of Oceanography, the PAN Department of Paleobiology, the PAN Institute of Parisitology, the Institute of Meteorology and Water Management, the Szczecin Agricultural Academy, Warsaw University and the Military Institute of Aviation Medicine.

The following problems were included among the main research projects:

--regional examinations of the geological structure of the South Shetland Archipelago using surface geology methods including various-scale photographs; stratographic, tectonic, sedimentological, paleontologic and paleoecologic investigations as well as exploratory geophysical methods (profile photographs using a proton magnetometer) using helicopters as a source of transportation and aerial reconnaissance. The more distant surrounding areas of the Arctowski Station were stipulated as the primary areas, that is the remaining and still uninvestigated areas of King George Island, Nelson Island and the western extremes of Livingston Island (Byers Peninsula).

The goal of these investigations was to examine the geological structure of the South Shetland Archipelago as a basis for drawing conclusions concerning the geological structure of the Antarctic shelf and the possibility of mineral resources;

--further examinations of the biological environment of Admiral Bay's water region on King George Island using biological (including parasitological), biochemical and oceanographic methods as well as investigations of its water region fringes (hydrochemical, biological and other methods), at times using air transport. Investigations encompassing the water regions of Admiral Bay were supposed to be done with the aid of research cutters. It was projected that special attention would be paid to problems associated with the functioning of the Antarctic's biological system, with special attention to investigating the processes of the flow of materials in the land-sea system. In addition, investigations on the possibility of obtaining nutritive protein from krill (Euphausia Superba) were stipulated;

--stationary geophysical (magnetism and seismology) and meteorological observations (in the latter case as a WMO meteorological shield and weather service;

--protecting the environment of the H. Arctowski Station: the effect of the Station's activities on the land and sea environment, the durability of the building materials and construction used there, the extent of accumulated contaminations and the like;

--the effect of this environment on the human organism.

The Scope of Technical work. The technical problems associated with the specific of the ground operations of the 1980-1981 summer expedition required the use of helicopters (then located at the Station) to transport the ground groups, especially the earth sciences and biology groups. Two Polish-made Mi-2 helicopters were used, which have been used successfully during the Third Expedition to the Arctowski Station (1978-1979). The plan called for the establishment of an auxiliary fuel base for the helicopters on Greenwich Island in Yankee Harbor Bay and then its elimination at the end of the summer season with the aid of the expedition ships. A expedition ship was supposed to try to land the sedimentological-paleontological ground group on Livingston Island, which was supposed to remain there about 1 month and then was flown via helicopter to the Arctowski Station. An alternative solution in case it was impossible to land on Livingston Island by ship because of poor weather and ice conditions and undesirable landing spots was to transport the group from the Arctowski Station to Livingston Island via two helicopters operating simultaneously.

In view of the reconstruction of the Arctowski Station during the Second and Third expeditions, no significant investments were planned except the building of a new magnetic pavillion. The expedition's technical operations plan stipulated mainly technical examinations, repairs, improving existing stationary and mobile equipment, certain adaptations and so forth.

The Scope of International Cooperation. Working and official visits to the scientific stations of King George Island were stipulated, that is to the Bellinghausen Station (USSR), the Presidente Frei Station (Chile) and Teniente Jubany Station (Argentina) using the expedition's helicopters. On the return trip to Poland, a visit to the Argentine and Chilean stations on Antarctic Peninsula to discuss conditions for closer cooperation in coming years was stipulated.

A Calendar of the More Important Events During the Expedition

1. The departure of the Fifth Expedition from Szczecin on 27 November 1980: 24 participants (including five from the winter group) aboard the M/S "Zulawy."

2. South Georgia (Gold Harbor), 22-28 December 1980: provisioning Polish fishing ships. Tests of expedition floating equipment (rubber pontoons and slips) along the M/S "Zulawy"'s mooring itinerary--the South Georgia coast (Gold Harbor).

3. East Falkland (Port William), 30 December 1980-5 January 1981. Provisioning Polish fishing ships. Tests of expedition (j.w.) floating equipment along the itinerary: mooring of the M/S "Zulawy" (Port William)--Port Stanley and other places on the island.

4. The arrival of the Fifth Expedition at Admiral Bay (King George Island), at the H. Arctowski Station, 8 January 1981 at 5:50. The transport of people and equipment to the Station completed 11 January 1981 at 08:00.

5. An attempt to land on Livingston Island (Byers Peninsula) the sedimentology-paleontology group (four people) together with a landing group from the M/S "Zulawy." A sudden worsening of the weather (wind and snow storms) which lasted 2 days forced termination of the operation and the return of the ship to the Arctowski Station (11-12 January 1981). The M/S "Zulawy" departed 12 January 1981 at 20:30 on a fishing trip.

6. Research and organizational-technical activity of the Fifth Expedition in the South Shelland region based at the Arctowski Station, 13 January to 23 March 1981: ground operations of the geology, geophysics and biology groups started 14 January;

--using a helicopter, ground operations of the named groups started 18 January and ended 15 March 1981. As a result of an engine failure on one of the helicopters (the lack of an engine cooling shield that was dismounted mistakenly by the aviation group together with the failed engine during the Third Expedition in 1979) it was not possible to use both helicopters simultaneously. That is why I was forced to cancel the planned geological-paleontologic research on Livingston Island because of flight safety on such a long journey; as a result, research work on King George and Nelson Islands was intensified.

7. From 27-29 January 1981, the Arctowski Station hosted the following ships of the FIBEX (BIOMASS) international research program: "Walter Herwis" (FRG), "Odysej" (USSR) and "Itzuma" (Chile) as well as the oceanographic ship "Meteor" (FRG). I made the Station available as a location for the FIBEX participants and participants of the oceanographic expedition from the ship "Meteor" visited the station.

8. From 4-7 February 1981 the S/Y "Pogoria" visited Arctowski Station, bringing two members of the Fifth Expedition's winter group and taking to Poland eight members of the Fourth Expedition's winter team.

9. On 7 and 16 March and from 21-24 March 1981 the Polish ship R/V "Professor Siedlecki" visited Admiral Bay's water regions, executing its program in the FIBEX framework. Its management and expedition participants visited the Station and for several days four of them worked at the Arctowski Station using its facilities and floating equipment.

10. The Arctowski Station also hosted the following research ships: "Yelcho" (Chile) on 24 January 1981 and "Hero" (United States) on 12 February and 16 March 1981 as well as the tourist ships: "World Discoverer" on 14 and 28 January 1981 and "Linblad Explorer" on 23 January and 4 February 1981; all in all about 600 foreign tourists visited the Station.

11. Official and working visits to the King George Island stations:

--Bellinghausen (USSR): 28-29 January 1981--five participants of the Fifth Expedition together with the management as well as two foreign guests (FRG), an exploratory visit; 19-24 February--four participants of the sedimentology-paleontology group visit the Soviet station doing research on the Fildes Peninsula; 19 February--the director of the Bellinghausen Station and three coleagues visited Arctowski Station; during March there were many working visits, including the delivery of spare parts for land vehicles at Bellinghausen Station, the familiarization of our participants with transferring fuel to the tank located at this station and so forth. All contacts occurred with the aid of Polish helicopters;

--Presidente Frei-Teniente Marsh (Chile): 20 January 1981--two Chilean helicopters visited the station management and last winter's crew (taking leave) and the new management together with 26 people; 28-29 January--another Polish visit to the Chilean station (our helicopter); 14 February--transported guests from the FRG to an airplane taking off for America using our helicopter; 19-24 February--a visit from our sedimentology-paleontology group living at the Bellinghausen Station, part of the Chilean station group; 4 March--the director of the Chilean station (O'Higgins Station) on the Antarctic Peninsula visited the Arctowski Station. In addition, using our helicopter, assistance was provided in retrieving a pontoon washed up by waves on the shore south of the Chilean station;

--Teniente Jubany (Argentina): 23 January 1981--an exploratory visit from us and help by Polish specialists in repairing heating equipment at the Argentine station; 28 January--transported food via our helicopters from the Teniente Jubany Station to the Argentine land base on Ardley Island; 28-29 January--four Argentine biologists revisited the Polish station, transported via our helicopter. In addition, there were many working visits during February and March to the Argentine station in conjunction with Polish land operations in its surrounding areas.

12. Return trip on 23 March 1981 at 10:00 to Admiral Bay by the expedition ship M/S "Antoni Garnuszewski". Twelve participants in the winter group visited Arctowski Station, bringing the number of winter group personnel to 19. The return trip of people, equipment, supplies and so forth lasted to 27 March. The winter group of the Fifth Expedition (including a part of the winter group of the Fourth Expedition), a total of 31 people, plus 19 people from the FIBEX Expedition embarked from the R/V "Professor Siedlecki."

13. On 28 March 1981 at 1:00 the M/S "Antoni Garnuszewski," commanded by Navy Capt Tadeusz Draczkowski, left Admiral Bay on the return trip to Poland. On the way it stopped at Cabedo, Brazil, (8-10 April) to take on cargo and in Las Palmas, Canary Islands (April 18). The expedition ended in Gdynia on 27 April 1981.

Main Results of the Scientific Research

Regional Geology (Professor K. Birkenmajer and team). Using a helicopter as a source of transport and at times with the aid of a research cutter, geological photographs on a 1:50,000 scale were taken of those portions of King George Island that were still unexplored, namely the area between Penguin Island and Cape Melville and farther on toward the north around North Foreland and then the entire north area of the island's shore between North Foreland and Fildes Peninsula together with numerous islets, and finally the entire Maxwell Bay region along with the eastern part of Nelson Island. The 1:50,000 scale geological photographs taken during the Fifth Expedition were of areas that measured over 1000 km^2.

In addition several very detailed maps were drawn up of selected areas, for example, Cape Melville, Potter Peninsula and Barton Peninsula. Extensive sea fauna were discovered, probably of the Cretaceous age, in a pulpy sedimentary series on Cape Melville which to date has been considered mistakenly by British researchers as quaternary lava and tuff. The occurrence of traces of the Antarctic continent's glaciation in this complex has been ascertained.

The stratography, tectonics, volcanic forms and metallic mineralization of King George and Nelson Islands mesozoic and kainozoic complex were examined, and the large pass faults between these islands were thoroughly investigated. The history of the quaternary age, dormant volcano Melville Peak was developed.

Over 100 orientated samples of lava were collected for petrographic, geochemical and radiometric research.

Sedimentology (Professor R. Gradzinski, Dr S. Porebski). The sedimentological environment of the tertiary marine-galcial deposits of the Pliocene Polonaise glaciation between Lions Rump and Low Head (King George Island) was investigated, and detailed sedimentological studies were made of the tertiary land-derived deposits on Fildes Peninsula. In addition the volcano-derived agglomerates on Fildes Peninsula and around Arctowski Station were investigated as well as the gravely beach deposits at Admiral Bay. The group used a helicopter for transportation.

Paleontology (Dr A. Gazdzicki, Dr R. Wrona and at times with the help of Professor K. Birkenmajer's group). An extensive selection of Pliocene marine invertebrates from the region between Admiral Bay and King George Bay was collected, also a large selection of marine invertebrates and several remains of vertebrates, probably from Cretaceous deposits of the Cape Melville region. Detail paleoecological studies were conducted in both regions.

The remains of fossil flora (leaves, kernels and wood) of the tertiary period continued to be collected in the Fildes Peninsula region, on Potter Peninsula, in Ezcurr Bay and at Point Hennequin on Admiral Bay.

Deserving special attention is the collection of erratic Cambrian period limestone from remains of coral-shaped Archaeocyathi derived from sea-glacial and continent-glacial deposits on King George Island brought in during the glaciation period by drifting ice mountains and glaciers from within the Antarctic continent. The group used a helicopter for transportation.

Geophysics. (A. Szymanski and team). Measurements were taken with a proton magnetometer of the glacial and shore profiles between Ezcurr Bay and Bransfield Straights. In the modeling region, south of Arctowski Station, detailed profiling using a proton magnetometer were based on precise geodetic matrices (T. Radomyski).

A new magnetic station was placed into service in the reconstructed pavillion; stationary magnetic measurements and magnetic recording were conducted regularly (A. Szymanski, Eng J. Komorowski) as well as seismic recording (J. Weiss). During the construction of the magnetic pavillion and during operations on the glacial area a helicopter was used.

Oceanography. (R. Marks). Systematic observations of the emission and dissemination of marine-origin aerosol was conducted; aerosol samples were collected and then analyzed microscopically and chemically; in addition, experimental investigations of the flow of aerosol mass per unit area was conducted. The measuring mast and test console were installed near the Arctowski Station lighthouse.

Meteorology. Meteorological observations were conducted continuously in the WMO world system according to the program for a highest order station which were transmitted daily to the Bellinghausen and President Frei Stations (Z. Rawa, Eng A. Maciazek). Satellite pictures were taken and analyzed, developing prognoses for helicopter flights, the R/V "Professor Siedlecki," ships of the Fifth Expedition and the Polish fishing fleet operating in the South Georgia and Falkland areas, and also for those foreign ships requesting it (for example, the FIBEX program, tourist ships) (Eng G. Marczak).

Biometeorology (Eng E. Moczydlowski). Stationary investigations from the previous season were continued at automatic measurement stations in the penguin colony near Arctowski Station. With the arrival of the earth sciences group, investigations were also conducted at forward positions in colonies of breeding penguins between Maxwell Bay and Cape Melville.

Hydrology and Biochemistry (Dr W. Lawacz, Dr S. Misiaszek). Since mid-February 1981 measurements of organic carbon content, salinity, oxygen content and temperature changes were initiated in the lakes surrounding Arctowski Station using 10 posts.

Ornithology. With the use of helicopter flights, research was expanded on the location and numbers of birds on King George Island. In this way, ornithological investigations of the entire island during the breeding season were

executed, which to date has not been possible. Of the 54 penguin colonies recorded during the Fifth Expedition, only 9 were known previously, several of them described decades ago and which were not visited by anyone since that time; this creates an exceptional opportunity for comparative research (Dr B. Jablonski). In addition, research continued on the dynamics of the growth of nestlings of selected petrel species (Fr A. Wasilewski) and investigating the population and ringing selected bird colonies (Dr W. Trivelpiece, Dr N. Volkman and Dr R. Butler, United States).

Mammology (Dr R. Halba). With the aid of a helicopter, changes in the seal population were investigated in the wide areas surrounding Admiral Bay, and biological material was collected to investigate environmental pollution. Additional chemical tests were conducted on samples collected last year. After Dr R. Halba departed on the "Pogoria," Dr B. Jablonski continued investigating the pinnipedian population of the entire King George Island area using helicopter flights with the geological photography group.

Botany. Up to 5 February 1981, photosynthesis and deep breathing processes of selected species of marine algae were investigated (R. Gutkowski). The primitive production of selected species of marine algae was investigated, ascertaining the high photosynthesis content, and samples were collected for further biochemical analyses (Dr R. Dawson and Dr W. Schramm, the FRG). A collection of moss and lichen was gathered from all the sites visited by the ornithological group (Dr B. Jablonski) during the helicopter flights with the geological photography group.

Krill Research (Professor E. Kolakonowski, Dr K. Lachowicz). Investigations were conducted on the technological usefullness of fresh krill (Euphausia superba) to obtain edible protein via the cold autoprotolysis method patented in Poland. It was shown that unfrozen krill can be successfully processed via this method into protein precipitate and concentrate that are free of solid carapace parts. The conditions for the production of edible concentrated protein obtained from krill were determined. Samples were prepared for further detailed tests in Poland.

Engineering and Environmental Protection. Sensing devices were mounted on the fuel tank's external jacket; an anemometer and a device to measure tank deformation during winter were installed (Eng L. Rosciszewski). Soil samples were collected using the shallow geological-engineering drilling method (W. Danowski, Eng K. Rolnicki) to evaluate the geotechnical conditions of Arctowski Station. Area-elevation photographs of the fuel tank region were taken (Eng T. Radomyski).

International Cooperation

As in past years, during the Fifth Expedition Arctowski Station maintained regular communication with the Soviet Bellinghausen station and the Chilean station, Presidente Frei, transmitting daily weather reports to the WMO system. Cooperation with these two stations as well as with the Argentine station Teniente Jubany also included mutual assistance and exchange of experiences in technical problems as well as the exchange of scientific personnel. In the framework of the scientific personnel exchange, four members of our expedition's

sedimentological-paleontological group lived for 5 days at the Soviet station conducting scientific research on the Fildes Peninsula, and four Argentine biologists stayed 2 days at the Arctowski Station. Two FRG biologists, Dr R. Dawson and Dr W. Schramm, spent 19 days with the Fifth Expedition. They conducted research in the area of primary production of marine algae. Three American ornithologists--Dr W. Trivelpiece, Dr N. Volkman and Dr R. Butler--spent 3 weeks with the expedition continuing their research of the 1977-1978 period.

Arctowski Station was the site of a working conference organized by Prof G. Hempel (FRG) during the visit (27-29 January 1981) of ships of the FIBEX sea expedition. Taking advantage of the presence of Prof Dr Meischner, University of Gottigen geologists, who directed the marine-geological program on the ship R/V "Meteor," a program on the geology of the sea of the South Shetland region was discussed. During the same time, in the course of a visit to the Presidente Frei station, the principles of Polish-Chilean geological cooperation in the field of radiometric dating of tertiary and Mezozoic lava of King George Island was discussed with Prof F. Herve of Santiago, Chile.

Arctowski Station also hosted other representatives of science, especially from the National Science Foundation and Office of Polar Programs (Washington, DC), including Doctor Williams (present director of biological programs); Dr G. Llano (previous director of biological programs); A. F. Betzel (director of oceanographic programs); Professor Dearborne (zoologist); Prof M. D. Turner (director of earth sciences programs); and Prof I. W. D. Dalziel, well-known Anartic geologists from Lamont Geological Observatory (Columbia University). The Fifth Expedition's director organized a geological trip to the area around Arctowski Station for the latter two scientists.

Close cooperation developed traditionally with the research and tourist ships that visited Arctowski Station. At the request of their captains, Arctowski Station transmitted to these ships weather-icing conditions in the sector from Bellinghausen Sea through Drake Strait to the Sea of Weddell based on satellite pictures taken by our own WESSA equipment. Ships of the Polish fishing fleet in the South Georgia and Falkland regions, ships of the M/S "Zulawy," S/Y "Pogoria" and M/S "Antoni Garnuszewski" expeditions in the course of their journeys through Antarctic waters, and the research ship R/V "Professor Siedlecki" and other FIBEX program ships received analogous information on a regular basis.

Most Important Scientific Accomplishments

Among the most important technical accomplishments of the Fifth Expedition worthy of mention is the construction of a new magnetic pavillion at the Arctowski Station, at times with the help of a helicopter which was used to transport building material. The biological laboratory was also expanded by one segment.

As is done every year, inspections and maintenance were executed, and adaptations to Station installations, to the radio station, heavy equipment and so forth were accomplished. About 140 helicopter flights were completed, a total

of over 80 flight hours. The damaged helicopter engine was changed two times. The research cutter "Dziunia" was inspected and repaired; the cutter "Slon" was launched and prepared for operation. Several dozen trips were made on Admiral Bay, primarily by the "Dziunia," to fish for krill, to transport cargo and to safeguard the underwater operations of FIBEX expedition frogmen (Doc K. Jazdzewski and team). The Gryf pontoons were operated (during the expedition as well as for transport and research purposes) on Admiral Bay and previously during the layover of the expedition ship M/S "Zulawy" near South Georgia (18 landings) and near East Falkland (32 landings).

PTS amphibians were used to unload the M/S "Zulawy" at the Arctowski Station (about 200 tons) and the reloading of the M/S Antoni Garnuszewski". Together with the research cutter and pontoons, they were used to fill the "Siedlecki" with 100 tons of drinkable water from the Itslian Valley water intake (Ezcurr Bay) and the "Pogoria" with 40 tons of drinkable water and 18 tons of fuel.

Sanitary Care and BHP [Industrial Safety and Hygiene]

Medical care was provided by Dr Med W. Nurkiewicz and Dr Med J. Rozynski, the Fourth Expedition winter group doctors who stayed over for the entire Fifth Expedition period. Dr Rozynski also continued the research program of the Fourth Expedition winter group up to the arrival of the "Pogoria"; he also conducted daily control preflight tests of the helicopter pilots.

During the Fifth Expedition, despite many land operations by the research groups, frequently working 30-40 kilometers from Arctowski Station, no accident occurred requiring hospitalization or major surgery. Mountain-glacial, station, marine, aviation, fire-fighting and other BHP instructions were developed and vigorously observed, and appropriate rescue services were trained at the Station. Contact with Arctowski Station's radio center was ensured by continuous monitoring during all helicopter flights and each time any floating unit went to sea. Flight logs, sea-journey logs and mountain-glacial journey logs beyond the Station area were kept daily. Ground groups had established times to contact the Station's radio center.

All expedition participants received detailed training in BHP during the course in Mikolajek (September 1980) as well as during the journey aboard the M/S "Zulawy" and landings at South Georgia and East Falkland and finally during the trips at Arctowski Station as well as at the Station itself and its surrounding area.

In several cases, the Fifth Expedition's medical service also provided general medical and dental aid to passengers and crews of tourist ships visiting Arctowski Station.

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RECENT SCIENTIFIC DEVELOPMENTS DESCRIBED

Laser Development, Production

Zielona Gora GAZETA LUBUSKA in Polish 31 Aug 82 p 7

[Article by (brj): "A Laser Produced in Zielona Gora? By All Means!"]

[Text] Light Amplification by Stimulated Emission of Radiation--this is an apparatus which amplifies microwaves of light by means of the stimulated emission of radiation. This apparatus has diverse applications, not excluding the military. With the aid of the laser, complicated eye operations and precision surgical cutting are conducted. Recently the Americans have been conducting research on constructing a laser device to combat an enemy's missiles.

Before such a complicated apparatus is produced, however, studies are continuing in the scientific laboratories on the laser's applications. Future scientists and practical workers must also acquaint themselves with the essential operation of this apparatus. It is for them specifically that simple lasers of modest capacities are being produced. I do not think that too many readers are aware that they are being made in Zielona Gora.

The plant producing the lasers is located on Fabryczna St and is called the Center for Research and Development on Research and Teaching Apparatus (COBRABID). It is precisely here, in adapted warehouse rooms, that a modest-sized team, under conditions which are almost hutlike, is making lasers which are intended for the science laboratories of higher schools. For several years a small portion of the amount produced has been earmarked for export. That means on order from the Kanuer Firm from West Berlin, from the materials entrusted to them, finishes lasers intended for spectral research studies. Similar lasers, with capacities of 6 milliwatts, are being produced for Poland itself.

In addition to this production COBRABID is also making tension recorders and digital integrators. At present the 40-person team is aiming to make static-pressure sensors. It is a well-known fact that the reform has compelled us to seek out new types of production. For there is a lack of parts which were never purchased for the devices. Therefore, production has had to

be discontinued on apparatus which relies on imported subsets. New products have to be introduced in its place.

The plant's production is not large, amounting to 12 million zlotys in toto. There is apprehension that this will decline. People are leaving because, in addition to the fact that the work here requires a great deal of knowledge and precision of execution, the pay is not the best.

But what does the laser look like? It is a long tube, filled with a helium-neon mixture, which, thanks to the appropriate apparatus, begins to radiate. The radiation is amplified and directed. For this purpose a whole system of various devices are attached which for a humanist are extremely complicated, though for a technical person they are very simple. And it is already possible to carry out research with the use of the laser. One can also perform various "miracles" such as holography, or the creation of three-dimensional images. At COBRABID they say that this is possible even in Zielona Gora.

New Economic Sciences Institute

Warsaw ZYCIE GOSPODARCZE in Polish No 33, 5 Sep 82 p 2

[Article: "The INE Is in Operation"]

[Text] In 1981 a new scientific outpost of the PAN [Polish Academy of Sciences] was appointed with a location in Warsaw--the INE [Institute of Economic Sciences], which began operating on a wide scale during the year 1982.

The institute conducts scientific-research work in the following fields:

--economic theory, particularly the political economic theory of socialism;

--analysis, forecasting, and strategic studies of Poland's socioeconomic development;

--fundamental systematic-institutional solutions for the functioning of a socialist economy;

--economic integration of the socialist countries;

--developmental trends of the world economy;

--contemporary directions in the development of economic thought and economic research studies on an international scale.

The INE has been assigned the following tasks, among others:

--development of theoretical thought in support of Poland's experience, that of the other socialist countries, as well as worldwide practice;

--linking together in scientific-research works economic aspects with sociological, political-science, ethical, cybernetic, and other aspects;

--improvement of scientific personnel;

--propagandizing Polish scientific thought and Polish practices on an international scale.

These tasks are being implemented at the institute's plants and scientific laboratories. A Scientific Council will begin functioning in the very near future. The institute is cooperating with other institutes and scientific outposts within Poland and abroad.

The INE's directorship is being handled by a PAN member Prof Dr Hab Jozef Pajestka. The deputy directors are Prof Dr Hab Janusz Gorski and Prof Dr Hab Witold Trzeciakowski, while the scientific secretary is Prof Dr Hab Barbara Pradecka.

The address of the INE is as follows: 00-901 Warsaw, Palace of Culture and Science, pok 2215, tel 20-86-95, as well as 00-441, Warsaw, Newelska St 6, tel 36-63-81.

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